



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING CONTINUATION/DIVISIONAL  
APPLICATION UNDER 37 C.F.R. § 1.53(b)



Box PATENT APPLICATION  
Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

This is a request for filing a ☐ continuation ☒ divisional application under 37 C.F.R.  
§ 1.53(b) of pending Application No. 08/487,550 filed on June 7, 1995, for HUMAN B7.1-  
SPECIFIC PRIMATIZED ANTIBODIES AND TRANSFECTOMAS EXPRESSING SAID  
ANTIBODIES, by the following named inventor(s):

- (a) Full Name Darrell R. ANDERSON
- (b) Full Name Peter BRAMS
- (c) Full Name Nabil HANNA
- (d) Full Name Bill SHESTOWSKY
- (e) Full Name Cheryl HEARD

- ☒ The entire disclosure of the prior application from which a copy of the oath or declaration is  
supplied herewith is considered as being part of the disclosure of the accompanying  
application and is hereby incorporated by reference therein.
- ☐ This application is being filed by less than all the inventors named in the prior application.  
In accordance with 37 C.F.R. 1.63(d)(2), the Commissioner is requested to delete the  
name(s) of the following person or persons who are not inventors of the invention being  
claimed in this application.

- (a) Full Name \_\_\_\_\_
- (b) Full Name \_\_\_\_\_
- (c) Full Name \_\_\_\_\_

1. ☒ Enclosed is a copy of the prior Application No. 08/487,550 as originally filed on  
June 7, 1995, including copies of the specification, claims, drawings and the  
executed oath or declaration as filed.
2. ☐ Enclosed is a revised prior application and a copy of the prior executed oath or  
declaration as filed. No new matter has been added to the revised application.

3. ☒ One statement(s) claiming small entity status ☐ are enclosed ☒ were filed in prior Application No. 08/487,550, filed on June 7, 1995.
4. ☒ The filing fee is calculated below ☒ and in accordance with the enclosed preliminary amendment:

CLAIMS					
	NO. OF CLAIMS		EXTRA CLAIMS	RATE	FEE
Basic Application Fee					\$760.00
Total Claims	6	MINUS 20 =	0	x \$18.00 =	0.00
Independent Claims	1	MINUS 3 =	0	x \$78.00 =	0.00
If multiple dependent claims are presented, add \$260.00					0.00
Total Application Fee					760.00
If small entity status is claimed, subtract 50% of Total Application Fee					380.00
Add Assignment Recording Fee of \$40.00 if Assignment document is enclosed					0.00
<b>TOTAL APPLICATION FEE DUE</b>					<b>380.00</b>

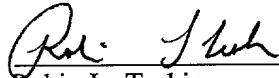
5. ☐ Charge \$ \_\_\_\_\_ to Deposit Account No. 02-4800 for the fee due.
6. ☒ A check in the amount of \$ 380.00 is enclosed for the fee due.
7. ☒ The Commissioner is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in triplicate.
8. ☒ Cancel in this application original claims 2-15 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)
9. ☒ Amend the specification by inserting before the first line the sentence: --This application is a ☐ continuation, ☒ divisional, of Application No. 08/487,550, filed June 7, 1995.--
10. ☐ Transfer the drawings from the pending prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate of this paper is

enclosed for filing in the prior application file. (May only be used if signed by person authorized under 37 C.F.R. § 1.138 and before payment of issue fee.)

11. ☒ New drawings are enclosed.
12. ☐ Priority of Application No. \_\_\_ filed on \_\_\_ in \_\_\_ (country) is claimed under 35 U.S.C. § 119.
- ☐ The certified copy of the priority application
- ☐ is enclosed
  - ☐ was filed on \_\_\_ in prior Application No. \_\_\_, filed on \_\_\_
  - ☐ has not yet been filed.
13. ☒ A preliminary amendment is enclosed.
14. ☒ Also enclosed Request for Listing of References Considered by Examiner and Statement Pursuant to 37 C.F.R. 1.822.
15. ☒ The power of attorney in the prior application is to E. Joseph Gess.
- a. ☒ The power appears in the original papers in the prior application.
  - b. ☐ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.
  - c. ☒ Recognize as Associate Attorney Robin L. Teskin, Reg. No. 35,030.
  - d. ☒ Address all future communications to: (May only be completed by applicant, or attorney or agent of record.)

E. Joseph Gess  
BURNS, DOANE, SWECKER & MATHIS, L.L.P.  
P.O. Box 1404  
Alexandria, Virginia 22313-1404

August 26, 1999  
Date

By:   
Robin L. Teskin  
Registration No. 35,030

ADDRESS OF  
SIGNATOR:

BURNS, DOANE, SWECKER & MATHIS, L.L.P.  
P.O. Box 1404  
Alexandria, Virginia 22313-1404  
(703) 836-6620

- ☐ inventor(s)
- ☐ assignee of complete interest
- ☐ attorney or agent of record
- ☒ filed under 37 C.F.R. § 1.34(a)

**COPY**

Patent  
Attorney's Docket No. 012712-131

Applicant or Patentee: Darrell R. ANDERSON et al

Application or Patent No.: 08/487,550

Filed or Issued: June 7, 1995

For: MONKEY MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN B7.1 AND/OR B7.2 PRIMATIZED FORMS THEREOF, PHARMACEUTICAL COMPOSITIONS CONTAINING, AND USE THEREOF AS IMMUNOSUPPRESSANTS

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS  
(37 C.F.R. §§ 1.9(f) AND 1.27(c)) - SMALL BUSINESS CONCERN**

I hereby declare that I am

- ☐ the owner of the small business concern identified below:  
☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN IDEC Pharmaceuticals Corporation

ADDRESS OF CONCERN 11011 Torrevana Road

San Diego, CA 92121

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 C.F.R. § 121.12, and reproduced in 37 C.F.R. § 1.9(d), for purposes of paying reduced fees under Sections 41(a) and 41(b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average, over the previous fiscal year of the concern, of the persons employed on a full-time, part-time, or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled MONKEY MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN B7.1 AND/OR B7.2 PRIMATIZED FORMS THEREOF, PHARMACEUTICAL COMPOSITIONS CONTAINING, AND USE THEREOF AS IMMUNOSUPPRESSANTS by inventor(s) Darrell R. ANDERSON, Peter BRAMS, Nabil HANNA, and Bill SHESTOWSKY, described in

- ☐ the specification filed herewith  
☒ Application No. 08/487,550, filed June 7, 1995  
☐ Patent No. \_\_\_\_\_, issued \_\_\_\_\_.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern, or organization having rights to the invention is listed below,\* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c), or by any concern that would not qualify as either a small business concern under 37 C.F.R. § 1.9(d) or a nonprofit organization under 37 C.F.R. § 1.9(e).

\*NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27.)

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

☐ individual      ☐ small business concern      ☐ nonprofit organization

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

☐ individual      ☐ small business concern      ☐ nonprofit organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earlier of the issue fee and any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b).)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Kenneth J. Woolcott

TITLE OF PERSON OTHER THAN OWNER Vice President, General Counsel & Licensing Exec.

ADDRESS OF PERSON SIGNING 11011 Torrevana Road, San Diego, CA 92121

SIGNATURE  DATE 11/21/95

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of )  
)  
Darrell R. ANDERSON et al. ) Group Art Unit: Unassigned  
)  
Application No. Unassigned ) Examiner: Unassigned  
)  
Filed: August 26, 1999 )  
)  
For: HUMAN B7.1-SPECIFIC PRIMATIZED )  
ANTIBODIES AND TRANSFECTOMAS )  
EXPRESSING SAID ANTIBODIES )

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination, please first amend the above-identified application as follows:

**IN THE SPECIFICATION**

Insert pages 59 to 80, containing the sequence listing for the subject application, and renumber the subsequent pages accordingly.

Page 21, line 1, delete "8a" and insert --8A-1 and 8A-2 (SEQ ID NOS:1-2)--;

Page 21, line 4, delete "8b" and insert --8B-1, 8B-2 and 8B-3 (SEQ ID NOS:3-4)--;

Page 21, line 7, delete "9a" and insert --9A-1 and 9A-2 (SEQ ID NOS:5-6)--;

Page 21, line 9, delete "9b" and insert --9B-1, 9B-2 and 9B-3 (SEQ ID NOS:7-8)--;

Page 21, line 11, delete "10a" and insert --10A-1 and 10A-2 (SEQ ID NOS:9-10)--; and

Page 21, line 13, delete "10b" and insert --10B-1, 10B-2 and 10B-3 (SEQ ID NOS:11-12)--.

**IN THE CLAIMS**

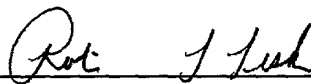
Claim 16, last line, please delete "any one of claims 1 to 11" and insert therefor --claim 1--.

**REMARKS**

Favorable consideration on the merits is respectfully requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By:   
Robin L. Teskin  
Registration No. 35,030

P.O. Box 1404  
Alexandria, Virginia 22313-1404  
(703) 836-6620

Date: August 26, 1999

TITLE OF THE INVENTION

5 MONKEY MONOCLONAL ANTIBODIES SPECIFIC TO  
HUMAN B7.1 AND/OR B7.2, PRIMATIZED FORMS THEREOF,  
PHARMACEUTICAL COMPOSITIONS CONTAINING,  
AND USE THEREOF AS IMMUNOSUPPRESSANTS

RELATED APPLICATIONS

There are no related applications.

FIELD OF THE INVENTION

669330-415860  
10 The present invention relates to the manufacture  
and identification of novel monoclonal antibodies to  
human B7, i.e., human B7.1 and human B7.2 and primatized  
forms thereof. More specifically, the present invention  
relates to the production and identification of macaque  
antibodies to human B7, i.e., human B7.1 and human B7.2  
15 produced by screening of phage display libraries and  
monkey heterohybridomas using B lymphocytes obtained  
from B7 immunized monkeys.

The invention further relates to specific  
primatized antibodies which bind to human B7, i.e.,  
20 human B7.1 and B7.2 as well as their corresponding amino  
acid and nucleic acid sequences.

Also, the present invention relates to  
pharmaceutical compositions containing monkey monoclonal  
or primatized antibodies specific to human B7.1 and/or  
25 human B7.2 and their use as immunosuppressants by  
modulating the B7:CD28 pathway, e.g., for the treatment  
of autoimmune disorders, and the prevention of organ  
rejection.

BACKGROUND OF THE INVENTION

30 The clinical interface between immunology,  
hematology, and oncology has long been appreciated.



Many conditions treated by the hematologist or oncologist have either an autoimmune or immunodeficient component to their pathophysiology that has led to the widespread adoption of immunosuppressive medications by hematologists, whereas oncologists have sought immunologic adjuvants that might enhance endogenous immunity to tumors. To date, these interventions have generally consisted of nonspecific modes of immunosuppression and immune stimulation. In addition to the limited efficacy of these interventions, toxicities secondary to their nonspecificity have also limited their overall success. Therefore, alternative strategies have been sought.

Elucidation of the functional role of a rapidly increasing number of cell surface molecules has contributed greatly to the integration of immunology with clinical hematology and oncology. Nearly 200 cell surface antigens have been identified on cells of the immune and hematopoietic systems (Schlossman SF, Boumsell L, Gilks JM, Harlan T, Kishimoto, C, Morimoto C, Ritz J, Shaw S, Silverstein RL, Springer TA, Tedder TF, Todd RF: CD antigens (1993), Blood 83:879, 1994). These antigens represent both lineage-restricted and more widely distributed molecules involved in a variety of processes, including cellular recognition, adhesion, induction and maintenance of proliferation, cytokine secretion, effector function, and even cell death. Recognition of the functional attributes of these molecules has fostered novel attempts to manipulate the immune response. Although molecules involved in cellular adhesion and antigen-specific recognition have previously been evaluated as targets of therapeutic immunologic intervention, recent attention has focused on a subgroup of cell surface molecules termed co-

stimulatory molecules (Bretscher P: "The two-signal model of lymphocyte activation twenty-one years later." Immunol. Today 13:73, (1992); Jenkins MK, Johnson JG: "Molecules involved in T-cell co-stimulation." Curr Opin Immunol 5:351, 1993; Geppert T, Davis L. Gur H. Wacholtz M. Lipsky P: "Accessory cell signals involved in T-cell activation." Immunol Rev 117:5, (1990); Weaver CT, Unanue ER: "The co-stimulatory function of antigen-presenting cells." Immunol Today 11:49, (1990); Stennam RM, Young JW: "Signals arising from antigen-presenting cells." Curr Opin Immunol 3:361, (1991)).

Co-stimulatory molecules do not initiate but rather enable the generation and amplification of antigen-specific T-cell responses and effector function

(Bretscher P: "The two-signal model of lymphocyte activation twenty-one years later." Immunol. Today 13:73, (1992); Jenkins MK, Johnson JG: "Molecules involved in T-cell co-stimulation." Curr Opin Immunol 5:351, (1993); Geppert T, Davis L. Gur H. Wacholtz M. Lipsky P: "Accessory cell signals involved in T-cell activation." Immunol Rev 117:5, (1990); Weaver CT, Unanue ER: "The co-stimulatory function of antigen-presenting cells." Immunol Today 11:49, (1990); Stennam RM, Young JW: "Signals arising from antigen-presenting cells." Curr Opin Immunol 3:361, (1991); June CH, Bluestone JA, Linsley PS, Thompson CD: "Role of the CD28 receptor in T-cell activation." Immunol Today 15:321, (1994)).

Recently, one specific co-stimulatory pathway termed B7:CD28 has been studied by different research groups because of its significant role in B and T cell activation (June CH, Bluestone JA, Linsley PS, Thompson CD: "Role of the CD28 receptor in T-cell activation." Immunol Today 15:321, (1994); June CH, Ledbetter JA:

"The role of the CD28 receptor during T-cell responses to antigen." Annu Rev Immunol 11:191, (1993); Schwartz RH: "Co-stimulation of T lymphocytes: The role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy." Cell 71:1065, (1992)). Since this ligand:receptor pathway was discovered four years ago, a large body of evidence has accumulated suggesting that B7:CD28 interactions represent one of the critical junctures in determining immune reactivity versus anergy (June CH, Bluestone JA, Linsley PS, Thompson CD: "Role of the CD28 receptor in T-cell activation." Immunol Today 15:321, (1994); June CH, Ledbetter JA: "The role of the CD28 receptor during T-cell responses to antigen." Annu Rev Immunol 11:191, (1993); Schwartz RH: "Co-stimulation of T lymphocytes: The role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy." Cell 71:1065, (1992); Cohen J: "Mounting a targeted strike on unwanted immune responses" (news; comment). Science 257:751, (1992); Cohen J: "New protein steals the show as 'co-stimulator' of T cells" (news; comment). Science 262:844, (1993)).

In particular, the role of the human B7 antigens, i.e., human B7.1 and B7.2, has been reported to play a co-stimulatory role in T-cell activation.

1. B7.1 and B7.2 Co-stimulatory Role in T Cell Activation

The elaboration of a successful immune response depends on a series of specific interactions between a T cell and an antigen presenting cell. Although the essential first step in this process depends upon the binding of antigen to the T cell receptor, in the context of the MHC class II molecule (Lane, P.J.L., F.M. McConnell, G.L. Schieven, E.A. Clark, and J.A.

- Ledbetter, (1990), "The Role of Class II Molecules in Human B Cell Activation." The Journal of Immunology, 144:3684-3692), this interaction alone is not sufficient to induce all the events necessary for a sustained
- 5 response to a given antigen (Schwartz, R.H. (1990), "A Cell Culture Model for T Lymphocyte Clonal Anergy." Science, 248:1349; Jenkins, M.K. (1992). "The Role of Cell Division in the Induction of Clonal Anergy." Immunology Today, 13:69; Azuma, M., M. Catabyab, D.
- 10 Buck, J.H. Phillips, and L.L. Lanier, (1992). "Involvement of CD28 in MHC-unrestricted Cytotoxicity Mediated by a Human Natural Killer Leukemia Cell Line." The Journal of Immunology, 149:1556-1561; Azuma, M., M. Catabyab, D. Buck, J.H. Phillips, and L.L. Lanier,
- 15 (1992). "CD28 Interaction with B7 Costimulates Primary Allogeneic Proliferative Responses and Cytotoxicity Mediated by Small Resting T Lymphocytes." J. Exp. Med., 175:353-360).

- The involvement of certain other co-stimulatory
- 20 molecules is necessary (Norton, S.D., L. Zuckerman, K.B. Urdahl, R. Shefner, J. Miller, and M.K. Jenkins. (1992), "The CD28 Ligand, B7, Enhances IL-2 Production by Providing A Costimulatory Signal to T Cells." The Journal of Immunology, 149:1556-1561). "The homodimers
- 25 CD28 and CTLA-4 expressed on T cells" (June, C.H., J.A. Ledbetter, P.S. Linsley, and C.B. Thompson, (1990), "Role of the CD28 Receptor in T-Cell Activation." Immunology Today, 11:211-216; Linsley, P.S., W. Brady, M. Urnes, L.S. Grosmaire, N.K. Damle, and J.A.
- 30 Ledbetter, (1991), "CTLA-4 is a Second Receptor for the B Cell Activation Antigen B7." J. Exp. Med., 174:561), together with B7.1 (CD80) and B7.2 (CD86) expressed on antigen presenting cells, are major pairs of co-stimulatory molecules necessary for a sustained immune

response (Azuma, M., H. Yssel, J.H. Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes." J. Exp. Med., 177:845-850; Freeman, G.J., A.S. Freedman, J.M. Segil, 5 G. Lee, J.F. Whitman, and L.M. Nadler, (1989), "B7, A New Member of the Ig Superfamily with Unique Expression on Activated and Neoplastic B Cells." The Journal of Immunology, 143:2714-2722; Hathcock, K.S., G. Laslo, H.B. Dickler, J. Bradshaw, P. Linsley, and R.J. Hodes, 10 (1993), "Identification of an Alternative CTLA-4 Ligand Costimulatory for T Cell Activation." Science, 262:905-911; Hart, D.N.J., G.C. Starling, V.L. Calder, and N.S. Fernando, (1993). "B7/BB-1 is a Leucocyte Differentiation Antigen on Human Dendritic Cells Induced 15 by Activation." Immunology, 79:616-620). It can be shown *in vitro* that the absence of these co-stimulatory signals leads to an aborted T cell activation pathway and the development of unresponsiveness to the specific antigen, or anergy. (See, e.g., Harding, F.A., J.G. 20 McArthur, J.A. Gross, D.M. Raullet, and J.P. Allison, (1992). "CD28 Mediated Signalling Co-sbmulates Murine T Cells and Prevents Induction of Anergy in T Cell Clones." Nature, 356:607-609; Gimmi, C.D., G.J. Freeman, J.G. Gribben, G. Gray, and L.M. Nadler, (1993). 25 "Human T-Cell Clonal Anergy is Induced by Antigen Presentation in the Absence of B7 Costimulation." Proc. Natl. Acad. Sci., 90:6586-6590; Tan, P., C. Anasefti, J.A. Hansen, J. Melrose, M. Brunvand, J. Bradshaw, J.A. Ledbetter, and P.S. Linsley, (1993), "Induction of 30 Alloantigen-specific Hyporesponsiveness in Human T Lymphocytes by Blocking Interaction of CD28 with Its Natural Ligand B7/BB1." J. Exp. Med., 177:165-173). Achievement of *in vivo* tolerance constitutes a mechanism for immunosuppression and a viable therapy for organ

transplant rejection and for the treatment of autoimmune diseases. This has been achieved in experimental models following the administration of CTLA4-Ig (Lenschow, D.J., Y. Zeng, R.J. Thistlethwaite, A. Montag, W. Brady, M.G. Gibson, P.S. Linsley, and J.A. Bluestone, (1992), "Long-Term Survival of Xenogeneic Pancreatic Islet Grafts Induced by CTLA-4Ig." Science, 257:789-795).

The molecules B7.1 and B7.2 can bind to either CD28 or CTLA-4, although B7.1 binds to CD28 with a Kd of 200 Nm and to CTLA-4 with a 20-fold higher affinity (Linsley, P.S., E.A. Clark, and J.A. Ledbetter, (1990), "T-Cell Antigen CD28 Mediates Adhesion with B Cells by Interacting with Activation Antigen B7/BB-1." Proc. Natl. Acad. Sci., 87:5031-5035; Linsley et al, (1993), "The Role of the CD28 receptor during T cell responses to antigen," Annu. Rev. Immunol., 11:191-192; Linesley et al, (1993), "CD28 Engagement by B7/BB-1 Induces Transient Down-Regulation of CD28 Synthesis and Prolonged Unresponsiveness to CD28 Signaling," The Journal of Immunology, 150:3151-3169). B7.2 is expressed on activated B cells and interferon induced monocytes, but not resting B cells (Freeman, G.J., G.S. Gray, C.D. Gimmi, D.B. Lomarrd, L-J. Zhou, M. White, J.D. Fingerioth, J.G. Gribben, and LM. Nadler, (1991). "Structure, Expression and T Cell Costimulatory Activity of the Murine Homologue of the Human B Lymphocyte Activation Antigen B7," J. Exp. Med., 174:625-631). B7.2, on the other hand, is constitutively expressed at very low levels on resting monocytes, dendritic cells and B cells, and its expression is enhanced on activated T cells, NK cells and B lymphocytes (Azuma, M. D. Ito, H. Yagita, K. Okumura, J.H. Phillips, L.L. Lanier, and C. Somoza, "1993", "B70 Antigen is a Second Ligand for CTLA-4 and CD28," Nature, 366:76-79). Although B7.1

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and B7.2 can be expressed on the same cell type, their expression on B cells occurs with different kinetics (Lenschow, D.J., G.H. Su, L.A. Zuckerman, N. Nabavi, C.L. Jellis, G.S. Gray, J. Miller, and J.A. Bluestone, (1993), "Expression and Functional Significance of an Additional Ligand for CTLA-4," Proc. Natl. Acad. Sci., USA, 90:11054-11058; Boussiotis, V.A., G.J. Freeman, J.G. Gribben, J. Daley, G. Gray, and L.M. Nadler, (1993), "Activated Human B Lymphocytes Express Three CTLA-4 Counter-receptors that Co-stimulate T-Cell Activation." Proc. Natl. Acad. Sci., USA, 90:11059-11063). Further analysis at the RNA level has demonstrated that B7.2 mRNA is constitutively expressed, whereas B7.1 mRNA is detected 4 hours after activation and initial low levels of B7.1 protein are not detectable until 24 hours after stimulation (Boussiotis, V.A., G.J. Freeman, J.G. Gribben, J. Daley, G. Gray, and L.M. Nadler, (1993), "Activated Human B Lymphocytes Express Three CTLA-4 Counter-receptors that Co-stimulate T-Cell Activation," Proc. Natl. Acad. Sci., USA, 90:11059-11063). CTLA-4/CD28 counter receptors, therefore, may be expressed at various times after B Cell activation.

The differential temporal expression of B7.1 and B7.2 suggests that the interaction of these two molecules with CTLA-4 and/or CD28 deliver distinct but related signals to the T cell (LaSalle, J.M., P.J. Tolentino, G.J. Freeman, L.M. Nadler, and D.A. Hafler, (1992), "CD28 and T Cell Antigen Receptor Signal Transduction Coordinately Regulate Intedeukin 2 Gene Expression In Response to Superantigen Stimulation," J. Exp. Med., 176:177-186; Vandenberghe, P., G.J. Freeman, L.M. Nadler, M.C. Fletcher, M. Kamoun, L.A. Turka, J.A. Ledbetter, C.B. Thompson, and C.H. June, (1992),

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5 "Antibody and B7/BB1-mediated Ligation of the CD28  
Receptor Induces Tyrosine Phosphorylation in Human T  
Cells," The Journal of Experimental Medicine,  
175:951-960). The exact signaling functions of CTLA-4  
and CD28 on the T cell are currently unknown (Janeway,  
C.A., Jr. and K. Bottomly, (1994), "Signals and Signs  
for Lymphocyte Responses," Cell, 76:275-285). However,  
it is possible that one set of receptors could provide  
the initial stimulus for T cell activation and the  
10 second, a sustained signal to allow further elaboration  
of the pathway and clonal expansion to take place  
(Linsley, P.S., J.L. Greene, P. Tan, J. Bradshaw, J.A.  
Ledbetter, C. Anasetti, and N.K. Damle, (1992),  
"Coexpression and Functional Cooperation of CTLA-4 and  
15 CD28 on Activated T Lymphocytes," J. Exp. Med.,  
176:1595-1604). The current data supports the two-  
signal hypothesis proposed by Jenkins and Schwartz  
(Schwartz, R.H., (1990), "A Cell Culture Model for T  
Lymphocyte Clonal Anergy," Science, 248:1349; Jenkins,  
20 M.K., (1992), "The Role of Cell Division in the  
Induction of Clonal Anergy," Immunology Today, 13:69)  
that both a TCR and co-stimulatory signal are necessary  
for T cell expansion, lymphokine secretion and the full  
development of effector function (Greenan, V. and G.  
25 Kroemer, (1993), "Multiple Ways to Cellular Immune  
Tolerance," Immunology Today, 14:573). The failure to  
deliver the second signal results in the inability of T  
cells to secrete IL-2 and renders the cell unresponsive  
to antigen.

30 Structurally, both B7.1 and B7.2 contain  
extracellular immunoglobulin superfamily V and C-like  
domains, a hydrophobic transmembrane region and a  
cytoplasmic tail (Freeman, G.J., J.G. Gribben, V.A.  
Boussiotis, J.W. Ng, V. Restivo, Jr., L.A. Lombard, G.S.



Gray, and L.M. Nadler, (1993), "Cloning of B7-2:  
A CTLA-4 Counter-receptor that Co-stimulates Human T  
Cell Proliferation," Science, 262:909). Both B7.1 and  
B7.2 are heavily glycosylated. B7.1 is a 44-54kD  
5 glycoprotein comprised of a 223 amino acid extracellular  
domain, a 23 amino acid transmembrane domain, and a 61  
amino acid cytoplasmic tail. B7.1 contains 3 potential  
protein kinase phosphorylation sites. (Azuma, M., H.  
Yssel, J.H. Phillips, H. Spits, and L.L. Lanier, (1993),  
10 "Functional Expression of B7/BB1 on Activated T  
Lymphocytes," J. Exp. Med., 177:845-850). B7.2 is a  
306 amino acid membrane glycoprotein. It consists of a  
220 amino acid extracellular region, a 23 amino acid  
hydrophobic transmembrane domain and a 60 amino acid  
15 cytoplasmic tail (Freeman, G.J., A.S. Freedman, J.M.  
Segil, G. Lee, J.F. Whitman, and LM. Nadler, (1989),  
"B7, A New Member of the Ig Superfamily with Unique  
Expression on Activated and Neoplastic B Cells," The  
Journal of Immunology, 143:2714-2722). Although both  
20 B7.1 and B7.2 genes are localized in the same  
chromosomal region (Freeman, G.J., D.B. Lombard, C.D.  
Gimmi, S.A. Brod, L Lee, J.C. Laning, D.A. Hafler, M.E.  
Dorf, G.S. Gray, H. Reiser, C.H. June, C.B. Thompson,  
and L.M. Nadler, (1992), "CTLA-4 and CD28 mRNA are  
25 Coexpressed in Most T Cells After Activation," The  
Journal of Immunology, 149:3795-3801; Schwartz, R.H.,  
(1992), "Costimulation of T Lymphocytes: The Role of  
CD28, CTLA-4, and B7/BB1" in Selvakumar, A., B.K.  
Mohanraj, R.L. Eddy, T.B. Shows, P.C. White, C. Perrin,  
30 and B. Dupont, (1992), "Genomic Organization and  
Chromosomal Location of the Human Gene Encoding the B-  
Lymphocyte Activation Antigen B7," Immunogenetics,  
36:175-181), these antigens do not share a high level of  
homology. The overall homology between B7.1 and B7.2 is

26% and between murine B7.1 and human S7 is 27% (Azuma, M., H. Yssel, J.H. Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes," J. Exp. Med., 177:845-850; Freeman, G.J.,  
5 A.S. Freedman, J.M. Segil, G. Lee, J.F. Whitman, and LM. Nadler, (1989), "B7, A New Member of the Ig Superfamily with Unique Expression on Activated and Neoplastic B Cells," The Journal of Immunology, 143:2714-2722).

Although alignment of human B7.1 human B7.2 and murine  
10 B.1 sequences shows few stretches of lengthy homology, it is known that all three molecules bind to human CTLA-4 and CD28. Thus, there is most likely a common, or closely homologous region shared by the three molecules that may be either contiguous or conformational. This  
15 region may constitute the binding site of the B7.1 and B7.2 molecules to their counter-receptors. Antibodies raised against these epitopes could potentially inhibit the interaction of B7 with its counter-receptor on the T cell. Furthermore, antibodies that cross-reacted with  
20 this region on both B7.1 and B7.2 molecules would potentially have practical advantages over antibodies directed against B7.1 or B7.2 separately.

## 2. Blockade of the B7/CD28 Interaction

Blocking of the B7/CD28 interaction offers the  
25 possibility of inducing specific immunosuppression, with potential for generating long lasting antigen-specific therapeutic effects. Antibodies to either B7.1 or B7.2 have been shown to block T cell activation, as measured by the inhibition of IL-2 production *in vitro* (DeBoer,  
30 M., P. Parren, J. Dove, F. Ossendorp, G. van der Horst, and J. Reeder, (1992), "Functional Characterization of a Novel Anti-B7 Monoclonal Antibody," Eur. Journal of Immunology, 22:3071-3075; Azuma, M., H. Yssel, J.H.

Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes," J. Exp. Med., 177:845-850). However, different antibodies have been shown to vary in their immunosuppressive

5 potency, which may reflect either their affinity or epitope specificity. CTLA-4/Ig fusion protein and anti-CD28 Fabs were shown to have similar effects on the down regulation of IL-2 production.

In vivo administration of a soluble CTLA-4/Ig

10 fusion protein has been shown to suppress T cell - dependent antibody responses in mice (Linsley, P.S., J.L. Greene, P. Tan, J. Bradshaw, J.A. Ledbetter, C. Anasetti, and N.K. Damle, (1992), "Coexpression and Functional Cooperation of CTLA-4 and CD28 on Activated T

15 Lymphocytes," J. Exp. Med., 176:1595-1604; Lin, H., S.F. Builing, P.S. Linsley, R.O. Wei, C.D. Thompson, and L.A. Turka, (1993), "Long-term Acceptance of Major Histocompatibility Complex Mismatched Cardiac Allografts Induced by CTLA-4-Ig Plus Donor Specific Transfusion,"

20 J. Exp. Med., 178:1801) and, furthermore, larger doses were also able to suppress responses to a second immunization, demonstrating the feasibility of this approach for the treatment of antibody mediated

25 autoimmune disease. In addition, CTLA-4/Ig was able to prevent pancreatic islet cell rejection in mice by directly inhibiting the interaction of T cells and B7.1/B7.2 antigen presenting cells (Lenschow, D.J., G.H. Su, L.A. Zuckerman, N. Nabavi, C.L. Jellis, G.S. Gray, J. Miller, and J.A. Bluestone, (1993), "Expression and

30 Functional Significance of an Additional Ligand for CTLA-4," Proc. Natl. Acad. Sci., USA, 90:11054-11058). In this case, long term donor specific tolerance was achieved.

### 3. Recombinant Phage Display Technology for Antibody Selection

To date, no monoclonal antibodies which crossreact with both B7.1 and B7.2 have been reported. As noted, such antibodies would potentially be highly desirable as immunosuppressants. Phage display technology is beginning to replace traditional methods for isolating antibodies generated during the immune response, because a much greater percentage of the immune repertoire can be assessed than is possible using traditional methods. This is in part due to PEG fusion inefficiency, chromosomal instability, and the large amount of tissue culture and screening associated with heterohybridoma production. Phage display technology, by contrast, relies on molecular techniques for potentially capturing the entire repertoire of immunoglobulin genes associated with the response to a given antigen.

This technique is described by Barber et al, Proc. Natl. Acad. Sci., USA, 88, 7978-7982, (1991). Essentially, immunoglobulin heavy chain genes are PCR amplified and cloned into a vector containing the gene encoding the minor coat protein of the filamentous phage M13 in such a way that a heavy chain fusion protein is created. The heavy chain fusion protein is incorporated into the M13 phage particle together with the light chain genes as it assembles. Each recombinant phage contains, within its genome, the genes for a different antibody Fab molecule which it displays on its surface. Within these libraries, in excess of  $10^6$  different antibodies can be cloned and displayed. The phage library is panned on antigen coated microliter wells, non-specific phage are washed off, and antigen binding phage are eluted. The genome from the antigen-specific clones is isolated and the gene III is excised, so that

antibody can be expressed in soluble Fab form for further characterization. Once a single Fab is selected as a potential therapeutic candidate, it may easily be converted to a whole antibody. A previously described expression system for converting Fab sequences to whole antibodies is IDEC's mammalian expression vector NEOSPLA. This vector contains either human gamma 1 or gamma 4 constant region genes. CHO cells are transfected with the NEOSPLA vectors and after amplification this vector system has been reported to provide very high expression levels (> 30 pg/cell/day) can be achieved.

#### 4. Primatized Antibodies

Another highly efficient means for generating recombinant antibodies is disclosed by Newman, (1992), Biotechnology, 10, 1455-1460. More particularly, this technique results in the generation of primatized antibodies which contain monkey variable domains and human constant sequences. This reference is incorporated by reference in its entirety herein. Moreover, this technique is also described in commonly assigned U.S. Application No. 08/379,072, filed on January 25, 1995, which is a continuation of U.S. Serial No. 07/912,292, filed July 10, 1992, which is a continuation-in-part of U.S. Serial No. 07/856,281, filed March 23, 1992, which is finally a continuation-in-part of U.S. Serial No. 07/735,064, filed July 25, 1991. 08/379,072 and the parent application thereof are incorporated by reference in their entirety herein.

This technique modifies antibodies such that they are not antigenically rejected upon administration in humans. This technique relies on immunization of cynomolgus monkeys with human antigens or receptors.

This technique was developed to create high affinity monoclonal antibodies directed to human cell surface antigens.

Antibodies generated in this manner have previously  
5 been reported to display human effector function, have  
reduced immunogenicity, and long serum half-life. The  
technology relies on the fact that despite the fact that  
cynomolgus monkeys are phylogenetically similar to  
10 humans, they still recognize many human proteins as  
foreign and therefore mount an immune response.  
Moreover, because the cynomolgus monkeys are  
phylogenetically close to humans, the antibodies  
generated in these monkeys have been discovered to have  
a high degree of amino acid homology to those produced  
15 in humans. Indeed, after sequencing macaque  
immunoglobulin light and heavy chain variable region  
genes, it was found that the sequence of each gene  
family was 85-98% homologous to its human counterpart  
(Newman et al, (1992), Id.). The first antibody  
20 generated in this way, an anti-CD4 antibody, was 91-92%  
homologous to the consensus sequence of human  
immunoglobulin framework regions. Newman et al,  
Biotechnology, 10:1458-1460, (1992).

Monoclonal antibodies specific to the human B7  
25 antigen have been previously described in the  
literature. For example, Weyl et al, Hum. Immunol.,  
31(4), 271-276, (1991) describe epitope mapping of human  
monoclonal antibodies against HLA-B-27 using natural and  
mutated antigenic variants. Also, Toubert et al, Clin.  
30 Exp. Immunol., 82(1), 16-20, (1990) describe epitope  
mapping of an HLA-B27 monoclonal antibody that also  
reacts with a 35-KD bacterial outer membrane protein.  
Also, Valle et al, Immunol., 69(4), 531-535, (1990)  
describe a monoclonal antibody of the IgG1 subclass

which recognizes the B7 antigen expressed in activated B cells and HTLV-1-transformed T cells. Further, Toubert et al, J. Immunol., 141(7), 2503-9, (1988) describe epitope mapping of HLA-B27 and HLA-B7 antigens using  
5 intradomain recombinants constructed by making hybrid genes between these two alleles in *E. coli*.

High expression of B7 antigen has been correlated to autoimmune diseases by some researchers. For example, Ionesco-Tirgoviste et al, Med. Interre, 24(1),  
10 11-17, (1986) report increased B7 antigen expression in type 1 insulin-dependent diabetes. Also, the involvement of B7 antigen expression on dermal dendritic cells obtained from psoriasis patients has been reported. (Nestle et al, J. Clin. Invest., 94(1), 202-  
15 209, (1994)).

Further, the inhibition of anti-HLA-B7 alloreactive CTL using affinity-purified soluble HLA-B7 has been reported in the literature. (Zavazava et al, Transplantation, 51(4), 838-42, (1991)). Further, the  
20 use of B7 receptor soluble ligand, CTLA-4-Ig to block B7 activity (See, e.g., Lenschow et al, Science, 257, 789, 7955 (1992)) in animal models and a B7-1-Ig fusion protein capable of inhibiting B7 has been reported.

#### SUMMARY AND OBJECTS OF THE INVENTION

25 An object of the invention is to produce and identify novel macaque antibodies to human B7 antigen, more specifically to human B7.1 antigen and/or human B7.2 antigen.

More specifically, it is an object of the present  
30 invention to produce and identify novel macaque antibodies to human B7 antigen, i.e., human B7.1 and human B7.2 antigen by screening of phage display libraries and/or monkey heterohybridomas using B

lymphocytes obtained from human B7 antigen, i.e., human B7.1 or B7.2 antigen immunized monkeys.

It is another specific object of the invention to provide anti-B7 monkey monoclonal antibodies and  
5 primatized forms thereof which specifically bind human B7.1 and/or B7.2 antigen which inhibit the B7/CD86 pathway and B7 stimulation of activated T cells, thereby inhibiting IL-2 production and T cell proliferation and functioning as effective immunosuppressants.

10 It is another object of the invention to provide anti-human B7.1 and anti-human B7.2 monkey monoclonal antibodies and primatized forms thereof which inhibit antigen driven responses in donor spleen cell cultures, e.g., antigen specific IgG responses, IL-2 production  
15 and cell proliferation.

It is another specific object of the invention to identify particular monkey monoclonal antibodies specific to human B7.1 and human B7.2 antigen and primatized forms thereof having advantageous properties,  
20 i.e., affinity, immunosuppressive activity, which are useful as therapeutics. More specifically, these monkey antibodies and primatized forms thereof are to be used, e.g., as immunosuppressants, i.e., to block antigen driven immune responses, to treat autoimmune diseases  
25 such as psoriasis, rheumatoid arthritis, systemic erythematosus (SLE), type 1 diabetes mellitus, idiopathic thrombocytopenia purpura (ITP), and to prevent organ rejection.

It is another object of the invention to provide  
30 pharmaceutical compositions containing one or more monkey monoclonal antibodies specific to human B7 antigen, i.e., human B7.1 and/or human B7.2 antigen, or primatized forms thereof, and a pharmaceutically acceptable carrier or excipient. These compositions



will be used, e.g., as immunosuppressants to treat autoimmune diseases, e.g., idiopathic thrombocytopenia purpura (ITP) and systemic lupus erythematosus (SLE), to block antigen driven immune responses, and to prevent organ rejection in transplant recipients.

It is another object of the invention to provide novel methods of therapy by administration of therapeutically effective amounts of one or more monkey or primatized monoclonal antibodies which specifically bind to B7 antigen, i.e., human B7.1 and/or B7.2 antigens. Such therapeutic methods are useful for treatment of diseases treatable by inhibition of the B7:CD28 pathway e.g., autoimmune diseases such as idiopathic thrombocytopenia purpura (ITP), systemic lupus erythematosus (SLE), type 1 diabetes mellitus, psoriasis, rheumatoid arthritis, multiple sclerosis, aplastic anemia, as well as for preventing rejection in transplantation subjects.

It is still another object of the invention to provide transfectants, e.g., CHO cells, which express at least the variable heavy and light domains of monkey monoclonal antibodies specific to the human B7.1 and/or B7.2 antigen.

It is another object of the invention to provide nucleic acid sequences which encode the variable heavy and/or light domains of monkey monoclonal antibodies specific to human B7.1 and/or human B7.2 antigen, and expression vectors which provide for the expression of primatized antibodies containing these nucleic acid sequences.

#### Definitions

The following terms are defined so that the invention may be more clearly understood.

Depleting antibody - an antibody which kills activated B cells or other antigen presenting cells.

Non-depleting antibody - an antibody which blocks the co-stimulatory action of B7 and T cell activating ligands CD28 and CTLA-4. Thus, it anergizes but does not eliminate the antigen presenting cell.

Primatized antibody - a recombinant antibody which has been engineered to contain the variable heavy and light domains of a monkey antibody, in particular, a cynomolgus monkey antibody, and which contains human constant domain sequences, preferably the human immunoglobulin gamma 1 or gamma 4 constant domain (or PE variant). The preparation of such antibodies is described in Newman et al, (1992), "Primatization of Recombinant Antibodies for Immunotherapy of Human Diseases: A Macaque/Human Chimeric Antibody Against Human CDH, Biotechnology, 10:1458-1460; also in commonly assigned 08/379,072 both of which are incorporated by reference in their entirety herein. These antibodies have been reported to exhibit a high degree of homology to human antibodies, i.e., 85-98%, display human effector functions, have reduced immunogenicity, and may exhibit high affinity to human antigens.

B7 antigens - B7 antigens in this application include, e.g., human B7, B7.1 and B7.2 antigens. These antigens bind to CD28 and/or CTLA-4. These antigens have a co-stimulatory role in T cell activation. Also, these B7 antigens all contain extracellular immunoglobulin superfamily V and C-like domains, a hydrophobic transmembrane region and a cytoplasmic tail. (See, Freeman et al, Science, 262:909, (1993)), and are heavily glycosylated.

Anti-B7 antibodies - Antibodies, preferably monkey monoclonal antibodies or primatized forms thereof, which

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the pMS vector used to screen recombinant immunoglobulin libraries produced against B7 displayed on the surface of filamentous phage which contains primers based on macaque immunoglobulin sequences.

Figure 2 depicts the NEOSPLA expression vector used to express the subject primatized antibodies specific to human B7.1 antigen.

Figure 3 depicts monkey serum anti-B7.1 titers directed against cell surface B7.1 on transfected CHO cells.

Figure 4 depicts inhibition of radiolabeled sB7.1 binding by SB7.1 affinity-purified monkey antibodies in the presence of unlabeled SB7 and Mab L307.4 murine anti-B7.1.

Figure 5 depicts inhibition of binding of radiolabeled monkey 135 and L3707.4 anti-B7.1 antibodies to B7 positive human SB cells by competition with affinity-purified SB7.1.

Figure 6 depicts inhibition of radiolabeled B7-Ig binding to activated human peripheral blood T cells by competing with unlabeled SB7.1 murine anti-B7.1 (L307.4) and monkey 1127 affinity purified serum antibodies.

Figure 7 depicts inhibition of IL-2 protein in mixed lymphocyte cultures by anti-B7.1 affinity-purified monkey serum antibodies.

Figure 8a depicts the amino acid and nucleic acid sequence of a primatized form of the light chain of 7C10.

5 Figure 8b depicts the amino acid and nucleic acid sequence of a primatized form of the heavy chain of 7C10.

Figure 9a depicts the amino acid and nucleic acid sequence of a primatized form of the light chain of 7B6.

10 Figure 9b depicts the amino acid and nucleic acid sequence of a primatized form of the heavy chain of 7B6.

Figure 10a depicts the amino acid and nucleic acid sequence of a primatized light chain 16C10.

Figure 10b depicts the amino acid and nucleic acid sequence of a primatized heavy chain 16C10.

15

#### DETAILED DESCRIPTION OF THE INVENTION

As described above, the present invention relates to the manufacture of novel monkey monoclonal antibodies which specifically bind human B7.1 and/or human B7.2 antigen, as well as primatized antibodies derived therefrom. These antibodies possess high affinity to human B7.1 and/or B7.2 and therefore may be used as immunosuppressants which inhibit the B7:CD86 pathway.

20 Preparation of monkey monoclonal antibodies will preferably be effected by screening of phage display libraries or by preparation of monkey heterohybridomas using B lymphocytes obtained from B7 (e.g., human B7.1 and/or B7.2) immunized monkeys.

25 As noted, the first method for generating anti-B7 antibodies involves recombinant phage display technology. This technique is generally described *supra*.

30 Essentially, this will comprise synthesis of recombinant immunoglobulin libraries against B7 antigen

displayed on the surface of filamentous phage and selection of phage which secrete antibodies having high affinity to B7.1 and/or B7.2 antigen. As noted *supra*, preferably antibodies will be selected which bind to both human B7.1 and B7.2. To effect such methodology, the present inventors have created a unique library for monkey libraries which reduces the possibility of recombination and improves stability. This vector, pMS, is described in detail *infra*, and is shown in Figure 1.

Essentially, to adopt phage display for use with macaque libraries, this vector contains specific primers for PCR amplifying monkey immunoglobulin genes. These primers are based on macaque sequences obtained while developing the primatized technology and databases containing human sequences.

Suitable primers are disclosed in commonly assigned 08/379,072 incorporated by reference herein.

The second method involves the immunization of monkeys, i.e., macaques, against human B7 antigen, preferably against human B7.1 and B7.2 antigen. The inherent advantage of macaques for generation of monoclonal antibodies is discussed *supra*. In particular, such monkeys, i.e., cynomolgus monkeys, may be immunized against human antigens or receptors.

Moreover, the resultant antibodies may be used to make primatized antibodies according to the methodology of Newman et al, Biotechnology, 10, 1455-1460, (1992), and Newman et al, commonly assigned U.S. Serial No. 08/379,072, filed January 25, 1995, which are incorporated by reference in their entirety.

The significant advantage of antibodies obtained from cynomolgus monkeys is that these monkeys recognize many human proteins as foreign and thereby provide for the formation of antibodies, some with high affinity to

desired human antigens, e.g., human surface proteins and cell receptors. Moreover, because they are phylogenetically close to humans, the resultant antibodies exhibit a high degree of amino acid homology to those produced in humans. As noted above, after sequencing macaque immunoglobulin light and heavy variable region genes, it was found that the sequence of each gene family was 85-88% homologous to its human counterpart (Newman et al, (1992), Id.).

Essentially, cynomolgus macaque monkeys are administered human B7 antigen, e.g., human B7.1 and/or human B7.2 antigen, B cells are isolated therefrom, e.g., lymph node biopsies are taken from the animals, and B lymphocytes are then fused with KH6/B5 (mouse x human) heteromyeloma cells using polyethylene glycol (PEG). Heterohybridomas secreting antibodies which bind human B7 antigen, e.g., human B7.1 and/or human B7.2 antigen, are then identified.

Antibodies which bind to both B7.1 and B7.2 are desirable because such antibodies potentially may be used to inhibit the interaction of B7.1 and B7.2, as well as B7 with their counter-receptors, i.e., human CTLA-4 and CD28. Antibodies against these epitopes may inhibit the interaction of both human B7.1 and human B7.2 with their counter receptors on the T cell. This may potentially provide synergistic effects.

However, antibodies which bind to only one of human B7 antigen, B7.1 antigen or B7.2 antigen, are also highly desirable because of the co-involvement of these molecules in T cell activation, clonal expansion lymphokine (IL-2) secretion, and responsiveness to antigen. Given that both human B7.1 and B7.2 bind to human CTLA-4 and CD28, it is probable that there is at least one common or homologous region (perhaps a shared

The present inventors elected to immunize macaques against human B7.1 antigen using recombinant soluble

5 B7.1 antigen produced in CHO cells and purified by  
affinity chromatography using a L307.4-sepharose  
affinity column. However, the particular source of  
human B7 antigen, human B7.1 antigen or human B7.2  
antigen is not critical, provided that it is of  
10 sufficient purity to result in a specific antibody  
response to the particular administered B7 antigen and  
potentially to other B7 antigens.

The human B7 antigen, human B7.1 antigen (also called CD80) and human B7.2 antigen (also called CD86) genes have been cloned, and sequenced, and therefore may readily be manufactured by recombinant methods.

Preferably, the administered human B7 antigen, human B7.1 antigen and/or human B7.2 antigen will be administered in soluble form, e.g., by expression of a B7, B7.1 or B7.2 gene which has its transmembrane and cytoplasmic domains removed, thereby leaving only the extracellular portion, i.e., the extracellular superfamily V and C-like domains. (See, e.g., Grumet et al, Hum. Immunol., 40(3), p. 228-234, 1994, which teaches expression of a soluble form of human B7, which is incorporated by reference in its entirety herein).

The macaques will be immunized with the B7, B7.1 and/or B7.2 antigen, preferably a soluble form thereof, under conditions which result in the production of antibodies specific thereto. Preferably, the soluble human B7, B7.1 or B7.2 antigen will be administered in combination with an adjuvant, e.g., Complete Freund's Adjuvant (CFA), Alum, Saponin, or other known adjuvants, as well as combinations thereof. In general, this will

require repeated immunization, e.g., by repeated injection, over several months. For example, administration of soluble B7.1 antigen was effected in adjuvant, with booster immunizations, over a 3 to 4 month period, with resultant production of serum containing antibodies which bound human B7.1 antigen.

After immunization B cells are collected, e.g., by lymph node biopsies taken from the immunized animals and B lymphocytes fused with KH6/B5 (mouse x human) heteromyeloma cells using polyethylene glycol. Methods for preparation of such heteromyelomas are known and may be found in U.S. Serial No. 08/379,072 by Newman et al, filed on January 25, 1995 and incorporated by reference herein.

Heterohybridomas which secrete antibodies which bind human B7, B7.1 and/or B7.2 are then identified. This may be effected by known techniques. For example, this may be determined by ELISA or radioimmunoassay using enzyme or radionuclide labelled human B7, B7.1 and/or B7.2 antigen.

Cell lines which secrete antibodies having the desired specificity to human B7, B7.1 and/or B7.2 antigen are then subcloned to monoclonality.

In the present invention, the inventors screened purified antibodies for their ability to bind to soluble B7.1 antigen coated plates in an ELISA assay, antigen positive B cells, and CHO transfectomas which express human B7.1 antigen on their cell surface. In addition, the antibodies were screened for their ability to block B cell/T cell interactions as measured by IL-2 production and tritiated thymidine uptake in a mixed lymphocyte reaction (MLR), with B7 binding being detected using <sup>125</sup>I-radiolabeled soluble B7.1 (SB7.1).



Also, affinity purified antibodies from macaques were tested for their reactivity against CHO transfectants which expressed B7.1/Ig fusion proteins, and against CHO cells which produced human B7.2 antigen.

5 These results indicated that the B7.1 immune sera bound to the B7.2 transfectomas. Binding of antibodies to B7.2 antigen may be confirmed using soluble B7.2-Ig reagents. As discussed in the examples, this may be effected by producing and purifying B7.2-Ig from CHO  
10 transfectomas in sufficient quantities to prepare a B7.2-Ig-sepharose affinity column. Those antibodies which cross-react with B7.2 will bind the B7.2-Ig-sepharose column.

Cell lines which express antibodies which  
15 specifically bind to human B7 antigen, B7.1 antigen and/or B7.2 antigen are then used to clone variable domain sequences for the manufacture of primatized antibodies essentially as described in Newman et al, (1992), Id. and Newman et al, U.S. Serial No. 379,072,  
20 filed January 25, 1995, both of which are incorporated by reference herein. Essentially, this entails extraction of RNA therefrom, conversion to cDNA, and amplification thereof by PCR using Ig specific primers. Suitable primers are described in Newman et al, 1992,  
25 Id. and in U.S. Serial No. 379,072. (See, in particular, Figure 1 of U.S. Serial No. 379,072).

The cloned monkey variable genes are then inserted into an expression vector which contains human heavy and light chain constant region genes. Preferably, this is  
30 effected using a proprietary expression vector of IDEC, Inc., referred to as NEOSPLA. This vector is shown in Figure 2 and contains the cytomegalovirus promoter/enhancer, the mouse beta globin major promoter, the SV40 origin of replication, the bovine growth

hormone polyadenylation sequence, neomycin  
phosphotransferase exon 1 and exon 2, human  
immunoglobulin kappa or lambda constant region, the  
dihydrofolate reductase gene, the human immunoglobulin  
5 gamma 1 or gamma 4 PE constant region and leader  
sequence. This vector has been found to result in very  
high level expression of primatized antibodies upon  
incorporation of monkey variable region genes,  
transfection in CHO cells, followed by selection in G418  
10 containing medium and methotrexate amplification.

For example, this expression system has been  
previously disclosed to result in primatized antibodies  
having high avidity ( $K_d \leq 10^{-10}$  M) against CD4 and other  
human cell surface receptors. Moreover, the antibodies  
15 have been found to exhibit the same affinity,  
specificity and functional activity as the original  
monkey antibody. This vector system is substantially  
disclosed in commonly assigned U.S. Serial No. 379,072,  
incorporated by reference herein as well as U.S. Serial  
20 No. 08/149,099, filed on November 3, 1993, also  
incorporated by reference in its entirety herein. This  
system provides for high expression levels, i.e., > 30  
pg/cell/day.

As discussed *infra*, the subject inventors have  
25 selected four lead candidate monkey monoclonal  
antibodies which specifically bind the B7.1 antigen, and  
which may also bind the B7.2 antigen. These monkey  
monoclonal antibodies are referred to herein as 7B6,  
16C10, 7C10 and 20C9.

30 As discussed in greater detail *infra*, these  
antibodies were evaluated for their ability to block B  
cell/T cell interactions as measured by IL-2 production  
and tritiated thymidine uptake in a mixed lymphocyte  
reaction for T cell binding experiments for T cell

binding, human body coat peripheral blood lymphocytes were cultured for 3-6 days in the presence of PHA stimulator. B7 binding was radioassayed using  $^{125}\text{I}$ -radiolabeled soluble B7.1. The observed results indicate that all of these antibodies bind B7.1 antigen with high affinity and effectively block B cell/T cell interactions as evidenced by reduced IL-2 production and reduced proliferation of mixed lymphocyte cultures.

The properties of these particular monkey monoclonal antibodies are summarized below:

1. To demonstrate the monkey antibodies' ability to block the physical interaction between CTLA4-Ig, varying concentrations of the monkey anti-B7.1 antibodies and unlabeled CTLA4-IG were incubated with radiolabeled CTLA4-Ig $^{125}$ . The results of the inhibition assay showed that the IC50 (the concentration of inhibitor which results in 50% inhibition) for the monkey antibodies are:

a:	7C10:	0.39 $\mu\text{g}/\text{Ml}$
b:	16C10:	1.60 $\mu\text{g}/\text{Ml}$
c:	20C9:	3.90 $\mu\text{g}/\text{Ml}$
d:	7B6:	39.0 $\mu\text{g}/\text{Ml}$

2. Scatchard analysis showed that the apparent affinity constants (Kd) for the monkey antibodies binding to B7-Ig coated plates were approximated to be:

a:	7C10:	$6.2 \times 10^{-9}\text{M}$
b:	16C10:	$8.1 \times 10^{-9}\text{M}$
c:	7B6:	$10.7 \times 10^{-9}\text{M}$
d:	20C9:	$16.8 \times 10^{-9}\text{M}$

3. The antibodies were tested *in vitro* in a mixed lymphocyte reaction assay (MLR). The MLR

showed that all 4 anti-B7.1 antibodies inhibit IL-2 production to different extents as shown by the following Ibgo values:

5           a:    7B6:        5.0 µg/M  
           b:    16C10:   <0.1 µg/M  
           c:    20C9:    2.0 µg/M  
           d:    7C10:    5.0 µg/M

- 10           4.    The monkey anti-B7.1 antibodies were tested for their ability to bind B7 on human peripheral blood lymphocytes (PBL). FACS analysis showed that all 4 monkey antibodies tested positive.
- 15           5.    Monkey antibodies 16C10, 7B6, 7C10 and 20C9 were tested for Clq binding by FACS analysis. Results showed 7C10 monkey Ig had strong human Clq binding after incubating with B7.1 CHO-transfected cells. 16C10 was positive, while 20C9 and 7B6 monkey antibodies were negative.
- 20           6.    To select an animal model for path-tox studies, the monkey antibodies were tested with animal blood from different species. It was determined that the monkey anti-B7.1 antibodies cross-reacted with human, chimpanzee, and possibly baboon.

25           Based on these properties, it would appear that three monkey monoclonal antibodies possess the most advantageous properties, 16C10, 7C10 and 20C9, with 16C10 and 7C10 being somewhat better than 20C9.

30           Using the techniques described *supra*, and in commonly assigned U.S. Serial No. 08/379,072, the present inventors have cloned the variable domains of 7C10, 7B6 and 16C10, and provide the amino acid and nucleic acid sequences of primatized forms of the 7C10 light chain, 7C10 heavy chain, 7B6 light chain, 7B6 heavy chain, 16C10 light chain and 16C10 heavy chain.

35

These amino acid and nucleic acid sequences may be found in Figures 8a and 8b, 9a and 9b, and 10a and 10b. The DNA and amino acid sequence for the human gamma 1 constant domain may be found in U.S. Serial No.

5 08/379,072.

As discussed *supra*, these primatized antibodies are preferably expressed using the NEOSPLA expression vector shown in Figure 2 which is substantially described in commonly assigned 08/379,072 and 08/149,099, both of which applications are incorporated by reference herein.

As previously noted, the subject primatized antibodies will preferably contain either the human immunoglobulin gamma 1 or gamma 4 constant region, with gamma 4 preferably mutated at two positions to create gamma 4 PE. The gamma 4 PE mutant contains two mutations, a glutamic acid in the CH2 region introduced to eliminate residual FCR binding, and a proline substitution in the hinge region, intended to enhance the stability of the heavy chain disulfide bond interaction. (See, Alegre et al, J. Immunol., 148, 3461-3468, (1992); and Angel et al, Mol. Immunol., 30, 105-158, (1993), both of which are incorporated by reference herein).

Whether the subject primatized antibodies contain the gamma 1, gamma 4 or gamma 4 PE constant region largely depends on the particular disease target. Preferably, depleting and non-depleting primatized IgG1 and IgG4 antibodies are created and tested against specific disease targets.

Given the described binding and functional properties of the subject monkey monoclonal antibodies, these anti-B7.1 monoclonal antibodies and primatized forms thereof should be well suited as therapeutic agents for blocking the B7:CD28 interaction thereby

porviding for immunosuppression. In particular, given their high affinity to B7.1 antigen and ability to block B cell/T cell interactions as measured by IL-2 production and tritiated thymidine uptake in mixed lymphocyte culture as well as their ability to effectively inhibit antigen driven responses in donor spleen cell cultures as shown by reduced antigen specific IgG responses, IL-2 production and cell proliferation, these monkey monoclonal antibodies and primatized forms thereof should function as effective immunosuppressants which modulate the B7:CD28 pathway. This is significant for the treatment of many diseases wherein immunosuppression is therapeutically desirable, e.g., autoimmune diseases, to inhibit undesirable antigen specific IgG responses, and also for prevention of organ rejection and graft-versus-host disease. Essentially, the subject antibodies will be useful in treating any disease wherein suppression of the B7:CD28 pathway is therapeutically desirable.

Key therapeutic indications for the subject anti-B7.1 antibodies include, by way of example, autoimmune diseases such as idiopathic thrombocytopenia purpura (ITP), systemic lupus erythematosus (SLE), type 1 diabetes mellitus, multiple sclerosis, aplastic anemia, psoriasis and rheumatoid arthritis.

Another significant therapeutic indication of the subject anti-B7.1 antibodies is for prevention of graft-versus-host-disease (GVHD) during organ transplant and bone marrow transplant (BMT). The subject antibodies may be used to induce host tolerance to donor-specific alloantigens and thereby facilitate engraftment and reduce the incidence of graft rejection. It has been shown in a murine model of allogeneic cardiac transplantation that intravenous administration of

CTLA4-Ig can result in immunosuppression or even induction of tolerance to alloantigen. (Lin et al, J. Exp. Med. 178:1801, 1993; Torka et al, Proc. Natl. Acad. Sci., USA, 89:11102, 1992). It is expected that the  
5 subject primatized anti-B7.1 antibodies will exhibit similar or greater activity.

Antibodies produced in the manner described above, or by equivalent techniques, can be purified by a combination of affinity and size exclusion  
10 chromatography for characterization in functional biological assays. These assays include determination of specificity and binding affinity as well as effector function associated with the expressed isotype, e.g., ADCC, or complement fixation. Such antibodies may be  
15 used as passive or active therapeutic agents against a number of human diseases, including B cell lymphoma, infectious diseases including AIDS, autoimmune and inflammatory diseases, and transplantation. The antibodies can be used either in their native form, or  
20 as part of an antibody/chelate, antibody/drug or antibody/toxin complex. Additionally, whole antibodies or antibody fragments (Fab<sub>2</sub>, Fab, Fv) may be used as imaging reagents or as potential vaccines or immunogens in active immunotherapy for the generation of anti-  
25 idiotypic responses.

The amount of antibody useful to produce a therapeutic effect can be determined by standard techniques well known to those of ordinary skill in the art. The antibodies will generally be provided by  
30 standard technique within a pharmaceutically acceptable buffer, and may be administered by any desired route. Because of the efficacy of the presently claimed antibodies and their tolerance by humans it is possible to administer these antibodies repetitively in order to

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combat various diseases or disease states within a human.

5 The anti-B7.1 antibodies (or fragments thereof) of this invention are useful for inducing immunosuppression, i.e., inducing a suppression of a human's or animal's immune system. This invention therefore relates to a method of prophylactically or therapeutically inducing immunosuppression in a human or other animal in need thereof by administering an effective, non-toxic amount of such an antibody of this invention to such human or other animal.

10 The ability of the compounds of this invention to induce immunosuppression has been demonstrated in standard tests used for this purpose, for example, a mixed lymphocyte reaction test or a test measuring inhibition of T-cell proliferation measured by thymidine uptake.

15 The fact that the antibodies of this invention have utility in inducing immunosuppression indicates that they should be useful in the treatment or prevention of resistance to or rejection of transplanted organs or tissues (e.g., kidney, heart, lung, bone marrow, skin, cornea, etc.); the treatment or prevention of autoimmune, inflammatory, proliferative and hyperproliferative diseases, and of cutaneous manifestations of immunologically mediated diseases (e.g., rheumatoid arthritis, lupus erythematosus, systemic lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type 1 diabetes, uveitis, nephrotic syndrome, psoriasis, atypical dermatitis, contact dermatitis and further eczematous dermatitides, seborrheic dermatitis, Lichen planus, Pemphigus, bullous pemphigus, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythema,



cutaneous eosinophilias, Alopecia areata, etc.); the treatment of reversible obstructive airways disease, intestinal inflammations and allergies (e.g., Coeliac disease, proctitis, eosinophilia gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis) and food-related allergies (e.g., migraine, rhinitis and eczema).

One skilled in the art would be able, by routine experimentation, to determine what an effective, non-toxic amount of antibody would be for the purpose of inducing immunosuppression. Generally, however, an effective dosage will be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

The antibodies (or fragments thereof) of this invention should also be useful for treating tumors in a mammal. More specifically, they should be useful for reducing tumor size, inhibiting tumor growth and/or prolonging the survival time of tumor-bearing animals. Accordingly, this invention also relates to a method of treating tumors in a human or other animal by administering to such human or animal an effective, non-toxic amount of an antibody. One skilled in the art would be able, by routine experimentation, to determine what an effective, non-toxic amount of anti-B7 antibody would be for the purpose of treating carcinogenic tumors. Generally, however, an effective dosage is expected to be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

The antibodies of the invention may be administered to a human or other animal in accordance with the aforementioned methods of treatment in an amount sufficient to produce such effect to a therapeutic or prophylactic degree. Such antibodies of the invention can be administered to such human or other animal in a

conventional dosage form prepared by combining the antibody of the invention with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables.

10 The route of administration of the antibody (or fragment thereof) of the invention may be oral, parenteral, by inhalation or topical. The term parenteral as used herein includes intravenous, intraperitoneal, intramuscular, subcutaneous, rectal or  
15 vaginal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred.

The daily parenteral and oral dosage regimens for employing compounds of the invention to prophylactically  
20 or therapeutically induce immunosuppression, or to therapeutically treat carcinogenic tumors will generally be in the range of about 0.05 to 100, but preferably about 0.5 to 10, milligrams per kilogram body weight per day.

25 The antibodies of the invention may also be administered by inhalation. By "inhalation" is meant intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may  
30 be prepared by conventional techniques. The preferred dosage amount of a compound of the invention to be employed is generally within the range of about 10 to 100 milligrams.

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The antibodies of the invention may also be administered topically. By topical administration is meant non-systemic administration and includes the application of an antibody (or fragment thereof) compound of the invention externally to the epidermis, to the buccal cavity and instillation of such an antibody into the ear, eye and nose, and where it does not significantly enter the blood stream. By systemic administration is meant oral, intravenous, intraperitoneal and intramuscular administration. The amount of an antibody required for therapeutic or prophylactic effect will, of course, vary with the antibody chosen, the nature and severity of the condition being treated and the animal undergoing treatment, and is ultimately at the discretion of the physician. A suitable topical dose of an antibody of the invention will generally be within the range of about 1 to 100 milligrams per kilogram body weight daily.

20 Formulations

While it is possible for an antibody or fragment thereof to be administered alone, it is preferable to present it as a pharmaceutical formulation. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, e.g., from 1% to 2% by weight of the formulation, although it may comprise as much as 10% w/w but preferably not in excess of 5% w/w and more preferably from 0.1% to 1% w/w of the formulation.

The topical formulations of the present invention, comprise an active ingredient together with one or more acceptable carrier(s) therefor and optionally any other therapeutic ingredients(s). The carrier(s) must be "acceptable" in the sense of being compatible with the

other ingredients of the formulation and not deleterious to the recipient thereof.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for  
5 penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

Drops according to the present invention may  
10 comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a  
15 surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 90°-100°C for half an hour. Alternatively, the solution may be sterilized by  
20 filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate  
25 (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An  
30 eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool

the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

5 Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of  
10 suitable machinery, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its  
15 derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogols. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surface active such as sorbitan  
20 esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous silicas, and other ingredients such as lanolin, may also be included.

25 The subject anti-B7.1 antibodies or fragments thereof may also be administered in combination with other moieties which modulate the B7:CD28 pathway. Such moieties include, by way of example, cytokines such as IL-7 and IL-10, CTLA4-Ig, soluble CTLA-4 and anti-CD28  
30 antibodies and fragments thereof.

It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of an antibody or fragment thereof of the invention will be determined by the nature and extent of

the condition being treated, the form, route and site of administration, and the particular animal being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill  
5 in the art that the optimal course of treatment, i.e., the number of doses of an antibody or fragment thereof of the invention given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination  
10 tests.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following formulations are,  
15 therefore, to be construed as merely illustrative embodiments and not a limitation of the scope of the present invention in any way.

#### Capsule Composition

A pharmaceutical composition of this invention in  
20 the form of a capsule is prepared by filling a standard two-piece hard gelatin capsule with 50 mg. of an antibody or fragment thereof of the invention, in powdered form, 100 mg. of lactose, 32 mg. of talc and 8 mg. of magnesium stearate.

#### 25 Injectable Parenteral Composition

A pharmaceutical composition of this invention in a form suitable for administration by injection is prepared by stirring 1.5% by weight of an antibody or fragment thereof of the invention in 10% by volume  
30 propylene glycol and water. The solution is sterilized by filtration.

Ointment Composition

Antibody or fragment thereof of the invention  
1.0 g.

White soft paraffin to 100.0 g.

- 5        The antibody or fragment thereof of the invention  
is dispersed in a small volume of the vehicle to produce  
a smooth, homogeneous product. Collapsible metal tubes  
are then filled with the dispersion.

Topical Cream Composition

- 10       Antibody or fragment thereof of the invention  
1.0 g.

Polawax GP 200 20.0 g.

Lanolin Anhydrous 2.0 g.

White Beeswax 2.5 g.

- 15       Methyl hydroxybenzoate 0.1 g.  
Distilled Water to 100.0 g.

- The polawax, beeswax and lanolin are heated  
together at 60°C. A solution of methyl hydroxybenzoate  
is added and homogenization is achieved using high speed  
20       stirring. The temperature is then allowed to fall to  
50°C. The antibody or fragment thereof of the invention  
is then added and dispersed throughout, and the  
composition is allowed to cool with slow speed stirring.

Topical Lotion Composition

- 25       Antibody or fragment thereof of the invention  
1.0 g.

Sorbitan Monolaurate 0.6 g.

Polysorbate 20 0.6 g.

Cetostearyl Alcohol 1.2 g.

- 30       Glycerin 6.0 g.

Methyl Hydroxybenzoate 0.2 g.

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Purified Water B.P. to 100-00 ml. (B.P. = British Pharmacopeia)

5 The methyl hydroxybenzoate and glycerin are dissolved in 70 ml. of the water at 75°C. The sorbitan monolaurate, polysorbate 20 and cetostearyl alcohol are melted together at 75°C and added to the aqueous solution. The resulting emulsion is homogenized, allowed to cool with continuous stirring and the antibody or fragment thereof of the invention is added  
10 as a suspension in the remaining water. The whole suspension is stirred until homogenized.

#### Eye Drop Composition

Antibody or fragment thereof of the invention  
0.5 g.  
15 Methyl Hydroxybenzoate 0.01 g.  
Propyl Hydroxybenzoate 0.04 g.  
Purified Water B.P. to 100-00 ml.

The methyl and propyl hydroxybenzoates are dissolved in 70 ml. purified water at 75°C and the  
20 resulting solution is allowed to cool. The antibody or fragment thereof of the invention is then added, and the solution is sterilized by filtration through a membrane filter (0.022  $\mu$ m pore size), and packed aseptically into suitable sterile containers.

#### 25 Composition for Administration by Inhalation

For an aerosol container with a capacity of 15-20 ml: mix 10 mg. of an antibody or fragment thereof of the invention with 0.2-0.5% of a lubricating agent, such as polysorbate 85 or oleic acid, and disperse such mixture  
30 in a propellant, such as freon, preferably in a combination of (1,2 dichlorotetrafluoroethane) and difluorochloro-methane and put into an appropriate



aerosol container adapted for either intranasal or oral inhalation administration.

Composition for Administration by Inhalation

For an aerosol container with a capacity of 15-20  
5 ml: dissolve 10 mg. of an antibody or fragment thereof  
of the invention in ethanol (6-8 ml.), add 0.1-0.2% of a  
lubricating agent, such as polysorbate 85 or oleic acid;  
and disperse such in a propellant, such as freon,  
preferably in combination of (1.2 dichlorotetra-  
10 fluoroethane) and difluorochloromethane, and put into an  
appropriate aerosol container adapted for either  
intranasal or oral inhalation administration.

The antibodies and pharmaceutical compositions of  
the invention are particularly useful for parenteral  
15 administration, i.e., subcutaneously, intramuscularly or  
intravenously. The compositions for parenteral  
administration will commonly comprise a solution of an  
antibody or fragment thereof of the invention or a  
cocktail thereof dissolved in an acceptable carrier,  
20 preferably an aqueous carrier. A variety of aqueous  
carriers may be employed, e.g., water, buffered water,  
0.4% saline, 0.3% glycine, and the like. These  
solutions are sterile and generally free of particulate  
matter. These solutions may be sterilized by  
25 conventional, well-known sterilization techniques. The  
compositions may contain pharmaceutically acceptable  
auxiliary substances as required to approximate  
physiological conditions such as Ph adjusting and  
buffering agents, etc. The concentration of the  
30 antibody or fragment thereof of the invention in such  
pharmaceutical formulation can vary widely, i.e., from  
less than about 0.5%, usually at or at least about 1% to  
as much as 15 or 20% by weight, and will be selected

primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention  
5 for intramuscular injection could be prepared to contain  
1 Ml sterile buffered water, and 50 mg. of an antibody  
or fragment thereof of the invention. Similarly, a  
pharmaceutical composition of the invention for  
intravenous infusion could be made up to contain 250 ml.  
10 of sterile Ringer's solution, and 150 mg. of an antibody  
or fragment thereof of the invention. Actual methods  
for preparing parenterally administrable compositions  
are well known or will be apparent to those skilled in  
the art, and are described in more detail in, for  
15 example, Remington's Pharmaceutical Science, 15th ed.,  
Mack Publishing Company, Easton, Pennsylvania, hereby  
incorporated by reference herein.

The antibodies (or fragments thereof) of the  
invention can be lyophilized for storage and  
20 reconstituted in a suitable carrier prior to use. This  
technique has been shown to be effective with  
conventional immune globulins and art-known  
lyophilization and reconstitution techniques can be  
employed.

25 Depending on the intended result, the  
pharmaceutical composition of the invention can be  
administered for prophylactic and/or therapeutic  
treatments. In therapeutic application, compositions  
are administered to a patient already suffering from a  
30 disease, in an amount sufficient to cure or at least  
partially arrest the disease and its complications. In  
prophylactic applications, compositions containing the  
present antibodies or a cocktail thereof are

administered to a patient not already in a disease state to enhance the patient's resistance.

Single or multiple administrations of the pharmaceutical compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical composition of the invention should provide a quantity of the altered antibodies (or fragments thereof) of the invention sufficient to effectively treat the patient.

It should also be noted that the antibodies of this invention may be used for the design and synthesis of either peptide or non-peptide compounds (mimetics) which would be useful in the same therapy as the antibody. See, e.g., Saragovi et al., Science, 253, 792-795 (1991).

To further illustrate the invention, the following examples are provided. These examples are not intended, nor are they to be construed, as further limiting the invention.

Example 1

Recombinant immunoglobulin libraries displayed on the surface of filamentous phage were first described by McCafferty et al, Nature, 348:552-554, 1990 and Barbas et al, Proc. Natl. Acad. Sci., USA 88:7978-7982, 1991. Using this technology, high affinity antibodies have been isolated from immune human recombinant libraries (Barbas et al, Proc. Natl. Acad. Sci., USA 89:10164-10168, 1992). Although the phage display concept used is substantially similar to that described by Barbas, 1991, Id. the technique has been modified by the substitution of a unique vector for monkey libraries to reduce the possibility of recombination and improve stability. This vector, pMS, Figure 1 contains a single

lac promoter/operator for efficient transcription and translation of polycistronic heavy and light chain monkey DNA. This vector contains two different leader sequences, the omp A (Movva et al, J. Biol. Chem., 255: 27-29, (1980), for the light chain and the pel B (Lei, J. Bact., 4379-109:4383 (1987) for the heavy chain Fd. Both leader sequences are translated into hydrophobic signal peptides that direct the secretion of the heavy and light chain cloned products into the periplasmic space. In the oxidative environment of the periplasm, the two chains fold and disulfide bonds form to create stable Fab fragments. We derived the backbone of the vector from the phagemid bluescript. (Stratagene, La Jolla, CA). It contains the gene for the enzyme beta-lactamase that confers ampicillin (carbenicillin) resistance to bacteria that harbor pMS DNA. We also derived, from bluescript, the origin of replication of the multicopy plasmid ColE1 and the origin of replication of the filamentous bacteriophage f1. The origin of replication of phage f1 (the so-called intragenic region), signals the initiation of synthesis of single stranded pMS DNA, the initiation of capsid formation and the termination of RNA synthesis by viral enzymes. The replication and assembly of pMS DNA strands into phage particles requires viral proteins that must be provided by a helper phage. We have used helper phage VCSM13 which is particularly suited for this, since it also contains a gene coding for kanamycin resistance. Bacteria infected with VCSM13 and pMS can be selected by adding both kanamycin and carbenicillin to the growth medium. The bacteria will ultimately produce filamentous phage particles containing either pMS or VCSM13 genomes. Packaging of the helper phage is less efficient than that of pMS, resulting in a mixed

phage population that contains predominately recombinant pMS phages. The ends of the phage pick up minor coat proteins specific to each end. Of particular interest here is the gene III product which is present in three  
5 to five copies at one end of the phage. The gene III product is 406 amino acid residues and is required for phage infection of *E. coli* via the F pili. The first two domains of the heavy chain, the variable and the CH1 domain, are fused to the carboxy-terminal half of the  
10 gene III protein. This recombinant pili protein, directed by the pel B leader, is secreted to the periplasm where it accumulates and forms disulfide bonds with the light chain before it is incorporated in the coat of the phage. Also, another vector contains a FLAG  
15 sequence engineered downstream of the gene III. The FLAG is an 8 amino acid peptide expressed at the carboxy terminal of the Fd protein. We are using commercially available monoclonal anti-FLAG M2 for both purification and detection of phage Fab by ELISA (Brizzard, Bio  
20 Techniques, 16(4):730-731, (1994)).

After constructing the vector pMS, we tested its ability to produce phage bound Fab using control antibody genes. We cloned an anti-tetanus toxoid antibody, (obtained from Dr. Carlos Barbas), into pMS  
25 and transformed XLI-blue. We co-infected our cells with VCSM13 and generated phage displaying the anti-tetanus toxoid antibody. We performed efficiency experiments where anti-tetanus toxoid phage were combined with phage beading an irrelevant antibody at 1:100,000. We  
30 performed three rounds of panning by applying 50  $\mu$ l of the mixed phage to antigen (tetanus toxoid) coated polystyrene wells. Non-adherent phage were washed off and the adherent phage were eluted with acid. The eluted phage were used to infect a fresh aliquot of XLI-

Blue bacteria and helper phage was added. After overnight amplification, phage were prepared and again panned on antigen coated plates. After three rounds of panning, we were able to show that we had successfully enriched for the anti-tetanus toxoid phage. The success of this technology also depends on the ability to prepare soluble Fabs for characterization of the final panned product. This was achieved by excising gene III from the pMS DNA using the restriction enzyme Nhe I followed by re-ligation. After the gene III was excised, the Fab was no longer displayed on the phage surface but accumulated in the periplasmic space. Lysates were prepared from bacteria expressing soluble Fab and tested for antigen specificity using an ELISA. High levels of soluble Fab were detected.

In order to adapt phage display technology for use with macaque libraries, we developed specific primers for PCR amplifying monkey immunoglobulin genes. These were based on macaque sequences we obtained while developing the PRIMATIZED™ antibody technology (See, 08/379,072, incorporated by reference herein) and databases containing human sequences. (Kabat et al, (1991), "Sequences of Proteins of Immunological Interest," U.S. Dept. of Health and Human Services, National Institute of Health).

We developed three sets of primers to cover amplification of the macaque repertoire. Our first set of primers was designed for amplification of the heavy chain VH and CH1 (Fd) domains. It consisted of a 3' CH1 domain primer and six 5' VH family specific primers that bind in the framework 1 region. Our second set of primers, for amplifying the whole lambda chain, covers the many lambda chain subgroups. It consists of a 3' primer and three 5' degenerate primers that bind in the

VL framework 1 region. Our third set of primers was designed for amplification of the kappa chain subgroups. It consists of one 3' primer and five VK framework 1 primers. Using each of these sets, PCR parameters were optimized to obtain strong enough signals from each primer pair so that ample material was available for cloning of the library. We recently created macaque combinatorial libraries in our pMS vector using these optimized PCR conditions. Bone marrow biopsies were taken from CD4 immune monkeys as the source of immunoglobulin RNA. The libraries contained approximately  $10^6$  members and are currently being panned for specific binders on antigen coated wells.

#### Example 2

##### 15 Development of B7/CTLA-4 Reagents

We have generated a number of reagents for the purpose of immunizing monkeys, developing binding and functional assays *in vitro*, screening heterohybridomas and panning phage libraries. Table 1 lists each reagent and its intended purpose. In the case of B7.1, RNA was extracted from SB cells and converted to cDNA using reverse transcriptase. The first strand cDNA was PCR amplified using B7.1 specific primers and cloned into IDEC's NEOSPLA mammalian expression vectors. CHO cells were transfected with B7.1 NEOSPLA DNA and clones expressing membrane associated B7.1 were identified. The B7.1 fusion protein was generated similarly, except that the PCR amplified B7.1 gene was cloned into a NEOSPLA cassette vector containing the human CH2 and CH3 immunoglobulin genes. CHO cells were transformed with the B7.1/Ig NEOSPLA DNA and stable clones secreting B7.1/Ig fusion protein were amplified. In general, the B7.2 and CTLA4 reagents were generated in the same

manner, except that for B7.2 the RNA was isolated from human spleen cells that had been stimulated 24 hours with anti-Ig and IL-4, and for the CTLA4 constructs the gene source was PHA activated human T cells.

5

**Table 1**

Reagent	Purpose	CHO Expression
Soluble B7.1	Immunization, immunoassays	Yes
B7.1 Transfectant	Screening, ELISA	Yes
B7.1/Ig Fusion Protein	Inhibition studies, panning	Yes
B7.2 Transfectant	Screening, ELISA	Yes
B7.2/Ig Fusion Protein	Inhibition studies, panning	To be completed
CTLA4 Transfectant	Inhibition studies	To be completed
CTLA4/Ig	Inhibition studies	To be completed

10

The availability of these reagents, together with monoclonal antibodies to B7.1 (L3074) (Becton Dickinson, 1994) and B7.2 (Fun-1 (Engel et al, Blood, 84, 1402-1407, (1994) and purified goat and rabbit antisera, specifically developed to detect monkey Fab fragments, facilitates identification of antibodies having the desired properties.

15

20

**Example 3**

**Investigation of the Immune Response in Cynomolgus Monkeys to Soluble and Cell Associated Human B7.1**

To evaluate the feasibility of producing monkey antibodies to human B7.1 antigen, we first purified recombinant SB7.1 from CHO cell media by affinity chromatography using a L307.4-sepharose affinity column. SB7.1 was then injected, with adjuvant, into five mature cynomolgus macaques. After a 3 to 4 month period of booster immunizations, sera from the monkeys immunized with SB7.1 or human SB cells were tested for antigen binding.

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Serum samples from the five monkeys immunized with SB7.1. and three additional animals immunized with B7.1 positive human SB cells, were tested for antibody titers against membrane associated B7.1 expressed in transfected CHO cells. The results summarized in Figure 3 showed that four out of five monkeys immunized with affinity-purified SB7.1 produced antibody titers in excess of 1:5000. The three animals immunized with SB cells containing cell associated B7.1 expressed lower titers of antibodies ranging from 1:1400 to 1:2800.

#### Example 4

We purified antibodies from sera of all eight immunized monkeys using SB7.1-sepharose and then tested their ability to bind to 1) SB7.1 coated plates in ELISA; 2) antigen positive B cells and 3) B7.1 CHO transfectomas. In addition, they were evaluated for their ability to block B cell interactions as measured by IL-2 production and tritiated thymidine uptake in a mixed lymphocyte reaction (MLR). For T cell binding experiments, human buffy coat peripheral blood lymphocytes were cultured for 3-6 days in the presence of PHA stimulator. B7 binding was detected by radio assay using  $^{125}\text{I}$ -radiolabeled soluble B7.1 (SB7.1).

#### Example 5

Direct binding of monkey antibodies to radiolabeled SB7.  $^{125}\text{I}$  radiolabeled SB7.1 was tested for binding to anti-B7.1 antibodies at 4, 1 and 0.25  $\mu\text{g/ml}$  in solution. The results shown in Table 2 suggest that most of the antibodies produced by monkeys immunized with SB7.1 were capable of binding the affinity-purified  $^{125}\text{I}$ -SB7.1 in a concentration dependent manner. To evaluate the specificity of binding to labeled SB7.1, unlabelled

concentration dependent manner. To evaluate the specificity of binding to labeled SB7.1, unlabelled SB7.1 competition experiments were done with antibodies from two animals. Affinity-purified antibodies from monkeys 1133 and 1144 were coated onto microwell plates at 400 ng/well. Affinity-purified unlabeled SB7.1 (500 and 100 ng/well) was used as competitor. The results shown in Figure 4 demonstrated that SB7.1 preparations are effective in inhibiting the  $^{125}\text{I}$ -SB7.1 from binding to the antibodies.

**Table 2**

**Binding of SB7-I $^{125}$  to Monkey Antibodies Affinity Purified on a SB7-Sepharose Affinity Column**

Antibody ( $\mu\text{g/ml}$ )	Monkey Numbers							
	769	908	1133	1135	1137	1139	1144	1146
4	175	213	9,056	12,771	4,318	226	5,781	108
1	106	142	6,569	7,940	3,401	110	3,901	80
0.25	95	104	1,803	2,673	1,219	100	1,186	94

Data are mean values of duplicate assays and represent cpm SB7-I $^{125}$  bound.

**Example 6**

**Direct binding of radiolabeled affinity-purified monkey antibodies to B7 $^{+}$  cells and inhibition by SB7.1.**

Affinity-purified radiolabeled monkey anti-B7.1 antibodies from monkey PRI135 were compared with radiolabeled L307.4 MAb for direct binding to B7 positive human SB cells. As a specificity control, unlabeled SB7.1 (0.002 - 20  $\mu\text{g/mi}$ ) was added to compete with both radiolabeled antibodies. We demonstrated that monkey antibodies can bind cell associated B7.1 and are inhibited with SB7.1, as shown in Figure 5. Inhibition as high as 90% was observed with SB7.1.

Example 7

Direct binding of radiolabeled B7-Ig fusion protein to activated T cells and Inhibition by affinity-purified monkey antibodies.

5 Human peripheral blood T lymphocytes were activated for 3-6 days and tested for direct binding of  $^{125}\text{I}$ -B7.1-Ig. Because of Fc receptor upregulation on activated human T cells, it was necessary to pre-incubate the cells with heat-aggregated pre-immune  
10 immunoglobulin to block Fc binding sites prior to addition of B7.1-Ig to the cells. A background control using SP2/0 murine myeloma cells was included to allow correction of the background binding. Figure 6 shows that inhibition of  $^{125}\text{I}$ -B7.1-Ig fusion protein binding to  
15 activated T cells was achieved with affinity-purified monkey antibodies at concentrations from 200 to 8  $\mu\text{g/ml}$ . Unlabeled SB7.1 and L307.4 MAb used as controls were also effective in inhibiting B7.1-Ig fusion protein cell binding.

Example 8

20 Inhibition of IL-2 production in mixed lymphocyte reactions by monkey anti-B7 antibodies.

The blocking of CD28/B7 interaction leads to inhibition of IL-2 production by T lymphocytes. In the  
25 experiment shown in Figure 7, affinity-purified monkey antibodies from two monkeys immunized with SB7.1 (monkeys 1137 and 1135) and one immunized with B7 positive SB cells (monkey 1146) were evaluated for their abilities to inhibit human T cell activation in mixed  
30 lymphocyte reaction (MLR), as measured by inhibition of IL-2 production. The results of this experiment show that affinity-purified anti-B7.1 antibodies from monkeys 1146 and 1137 inhibited IL-2 production when added at concentrations of 50  $\mu\text{g/ml}$ . Monkey 1135 antibodies

could not be evaluated at the two highest concentrations due to lack of material, yet gave significant inhibition at lower concentrations. The murine MAb L307.4 was inhibitory at concentrations of 10  $\mu$ g/ml. Other monkey sera tested at these concentrations were negative (data not shown). These results demonstrate that at least three of the monkeys immunized with both soluble and membrane associated forms of the B7 antigen are producing B7-blocking antibodies with immunosuppressive potential.

#### Example 9

##### **Investigation of Cross-reactivity in B7.1 Immunized Monkey Serum to B7.2 Antigen.**

Antibodies raised against B7.1 are to be tested for cross-reactivity to B7.2. Preliminary results using B7.1 affinity-purified antibodies from B7.1 immune sera provided suggestive evidence of binding to B7.2 transfected CHO cells (not shown). These data should be confirmed by using soluble B7.2Ig reagents. We will first purify additional monkey antibodies from B7.1 immunized animals by affinity chromatography on B7.1Ig--sepharose. We will then produce and purify B7.2Ig from CHO cells in sufficient quantities to prepare a B7.2Ig-sepharose affinity column. We will select from the B7.1 specific antibody population those antibodies which cross-react with B7.2 by binding to the B7.2Ig-sepharose column. Any cross-reactive antibodies identified will be further characterized by direct binding to both B7.1 and B7.2 transfected CHO cells and inhibition of binding to B7.2 transfected cells by B7.1Ig.

Example 10

**Generation of a Phage Display Library**

Recombinant phage display libraries are generated from B7.1 and B7.2 immune monkeys. Lymph node and bone marrow biopsies are performed 7-12 days after immunization to harvest RNA rich B cells and plasma cells. RNA is isolated from the lymphocytes using the method described by Chomczynski Anal. Biochem., 162(1), 156-159, (1987). RNA is converted to cDNA using an oligo dT primer and reverse transcriptase. The first strand cDNA is divided into aliquots and PCR amplified using the sets of kappa, lambda, and heavy chain Fd region primers described earlier and either Pfu polymerase (Stratagene, San Diego) or Taq polymerase (Promega, Madison). The heavy chain PCR amplified products are pooled, cut with Xho VSpe I restriction enzymes and cloned into the vector pMS. Subsequently, the light chain PCR products are pooled, cut with Sac I/Xba I restriction enzymes, and cloned to create the recombinant library. XLI-Blue *E. coli* is transformed with the library DNA and super-infected with VCSM13 to produce the phage displaying antibodies. The library is panned four rounds on polystyrene wells coated with B7.1 or B7.2 antigen. Individual phage clones from each round of panning are analyzed. The pMS vector DNA is isolated and the gene III excised. Soluble Fab fragments are generated and tested in ELISA for binding to B7.1 and B7.2.

Example 11

**30 Characterization of Phage Fab Fragments**

The monkey phage Fab fragments are characterized for their specificity and the ability to block B7.1-Ig and B7.2-Ig binding to CTLA-4-Ig or CTLA-4 transfected

cells. Phage fragments are also characterized for cross-reactivity after first panning for 4 rounds on the B7 species used for immunization in order to select for high affinity fragments. Fab fragments identified from  
5 four rounds of panning either on B7.1 or B7.2 antigen coated surfaces are scaled up by infection and grown in 24 hour fermentation cultures of E coli. Fragments are purified by Kodak FLAG binding to a anti-FLAG affinity column. Purified phage Fabs are tested for affinity by  
10 an ELISA based direct binding modified Scatchard analysis (Kato et al, J. Chem. BioEng., 76:451-454, (1993)) using Goat anti-monkey Fab antibodies or anti-FLAG MAb conjugated with horseradish peroxidase. The anti-monkey Fab reagents will be absorbed against human  
15 heavy chain constant region Ig to remove any cross-reactivity to B7-Ig. Kd values are calculated for each fragment after measurements of direct binding to B7.1-Ig or B7.2-Ig coated plates.

#### Example 12

##### 20 **Phage Fab Fragment Blocking of CTLA-4/B7 Binding**

Fab fragments most effectively blocking the binding of B7-Ig at the lowest concentrations are selected as lead candidates. Selections are made by competing off <sup>125</sup>I-B7-Ig binding to CTLA-4-Ig or CTLA-4 transfected  
25 cells. Additional selection criteria include, blocking of mixed lymphocyte reaction (MLR), as measured by inhibiting 3H-thymidine uptake in responder cells (Azuma et al, J. Exp. Med., 177:845-850,; Azuma et al, Nature, 301:76-79, (1993)) and direct analysis of IL-2  
30 production using IL-2 assay kits. The three or four candidates which are most effective in inhibiting of MLR and CTLA-4 binding assays are chosen for cloning into the above-described mammalian expression vector for

transfection into CHO cells and expression of chimeric monkey/human antibodies.

**Example 13**

**Generation of Monkey Heterohybridomas**

5 Monkey heterohybridomas secreting monoclonal  
antibodies are generated from existing immunized animals  
whose sera tested positive for B7.1 and/or B7.2. Lymph  
node biopsies are taken from animals positive to either,  
or both, antigens. The method of hybridoma production  
10 is similar to the established method used for the  
generation of monkey anti-CD4 antibodies (Newman,  
1992(Id.)). Monkeys with high serum titers will have  
sections of inguinal lymph nodes removed under  
anesthesia. Lymphocytes are washed from the tissue and  
15 fused with KH6/B5 heteromyeloma cells (Carrol et al, J.  
Immunol. Meth., 89:61-72, (1986)) using polyethylene  
glycol (PEG). Hybridomas are selected on H.A.T. media  
and stabilized by repeated subcloning in 96 well plates.

20 Monkey monoclonal antibodies specific for B7.1  
antigen are screened for cross-reactivity to B7.2.  
Monkey anti-B7 antibodies will be characterized for  
blocking of B7/CTLA-4 binding using the <sup>125</sup>I-B7-Ig  
binding assay. Inhibition of MLR by 3H-Thymidine uptake  
and direct measurement of IL-2 production is used to  
25 select three candidates. Two candidates will be brought  
forward in Phase II studies and expressed in CHO cells  
while repeating all functional studies. For the  
purposes of developing an animal model for *in vivo*  
pharmacology, anti-B7 antibodies will be tested on cells  
30 of several animal species. The establishment of an  
animal model will allow preclinical studies to be  
carried out for the selected clinical indication.

Example 14

As discussed *supra*, using the above heterohybridoma methods, 4 lead monkey anti-B7.1 antibodies have been identified: 16C10, 7B6, 7C10 and 20C9. These antibodies were characterized as follows:

To demonstrate the monkey antibodies' ability to block the physical interaction between CTLA4-Ig, varying concentrations of the monkey anti-B7.1 antibodies and unlabeled CTLA4-Ig were incubated with radiolabeled CTLA4-Ig<sup>I125</sup>. The results of the inhibition assay showed that the IC50 (the concentration of inhibitor which results in 50% inhibition) for the monkey antibodies are:

a:	7C10:	0.39 µg/Ml
b:	16C10:	1.60 µg/Ml
c:	20C9:	3.90 µg/Ml
d:	7B6:	39.0 µg/Ml

Scatchard analysis showed that the apparent affinity constants (Kd) for the monkey antibodies binding to B7-Ig coated plates were approximated to be:

a:	7C10:	$6.2 \times 10^{-9}M$
b:	16C10:	$8.1 \times 10^{-9}M$
c:	7B6:	$10.7 \times 10^{-9}M$
d:	20C9:	$16.8 \times 10^{-9}M$

The antibodies were tested *in vitro* in a mixed lymphocyte reaction assay (MLR). The MLR showed that all 4 anti-B7.1 antibodies inhibit IL-2 production to different extents:

a:	7B6:	5.0 µg/Ml
b:	16C10:	0.1 µg/Ml
c:	20C9:	2.0 µg/Ml
d:	7C10:	5.0 µg/Ml

The monkey anti-B7.1 antibodies were tested for their ability to bind B7 on human peripheral blood



lymphocytes (PBL). FACS analysis showed that all 4 monkey antibodies tested positive.

5 Monkey antibodies 16C10, 7B6, 7C10 and 20C9 were tested for C1q binding by FACS analysis. Results showed 7C10 monkey Ig had strong human C1q binding after incubating with B7.1 CHO-transfected cells. 16C10 was negative, as were the 20C9 and 7B6 monkey antibodies.

Example 15

10 Using the primatized antibody methodology incorporated by reference to commonly assigned U.S. Serial No. 08/379,072, and using the NEOSPLA vector system shown in Figure 2, the heavy and light variable domains of 7C10, 7B6 and 16C10 were cloned and  
15 primatized forms thereof have been synthesized in CHO cells using the NEOSPLA vector system. The amino acid and nucleic acid sequences for the primatized 7C10 light and heavy chain, 7B6 light and heavy chain, and 16C10 light and heavy chain are respectively shown in Figures  
20 8a, 8b, 9a, 9b, 10a and 10b.

It is expected that these primatized antibodies, given their probable low antigenicity and human effector function, will be well suited as therapeutics. In fact, it has recently been shown that primatized 16C10  
25 exhibits human C1<sub>q</sub> binding, whereas 16C10 does not.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments of the invention described herein. Such equivalents are  
30 intended to be embraced by the following claims.

What Is Claimed Is:

1. A monkey monoclonal antibody or a primatized form thereof which specifically binds human B7.1 antigen and/or human B7.2 antigen.

2. The antibody of claim 1 which is selected from the group consisting of 16C10, 7C10, 20C9 and 7B6.

3. The antibody of claim 1 which is a depleting antibody.

4. The antibody of claim 1 which is a non-depleting antibody.

5. A primatized antibody which specifically binds to human B7.1 antigen which contains the variable heavy and light domains of an antibody selected from the group consisting of 16C10, 7C10, 20C9 and 7B6.

6. The primatized antibody of claim 5 wherein said antibody is derived from 7C10 and has the amino acid sequence set forth in Figures 8a and 8b.

7. The primatized antibody of claim 6 which is encoded by the nucleic acid sequence set forth in Figures 8a and 8b.

8. The primatized antibody of claim 5 wherein said antibody is derived from 7B6 and has the amino acid sequence set forth in Figures 9a and 9b.

9. The primatized antibody of claim 8 wherein said antibody is encoded by the nucleic acid sequence set forth in Figures 9a and 9b.

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17. The method of claim 16 wherein said antibody is 16C10, 7C10, 20C9, 7B6 or a primatized form thereof.

19. The method of claim 16 wherein said disease is selected from idiopathic thrombocytopenia purpura, systemic lupus erythematosus, type 1 diabetes mellitus, rheumatoid arthritis, psoriasis and multiple sclerosis.

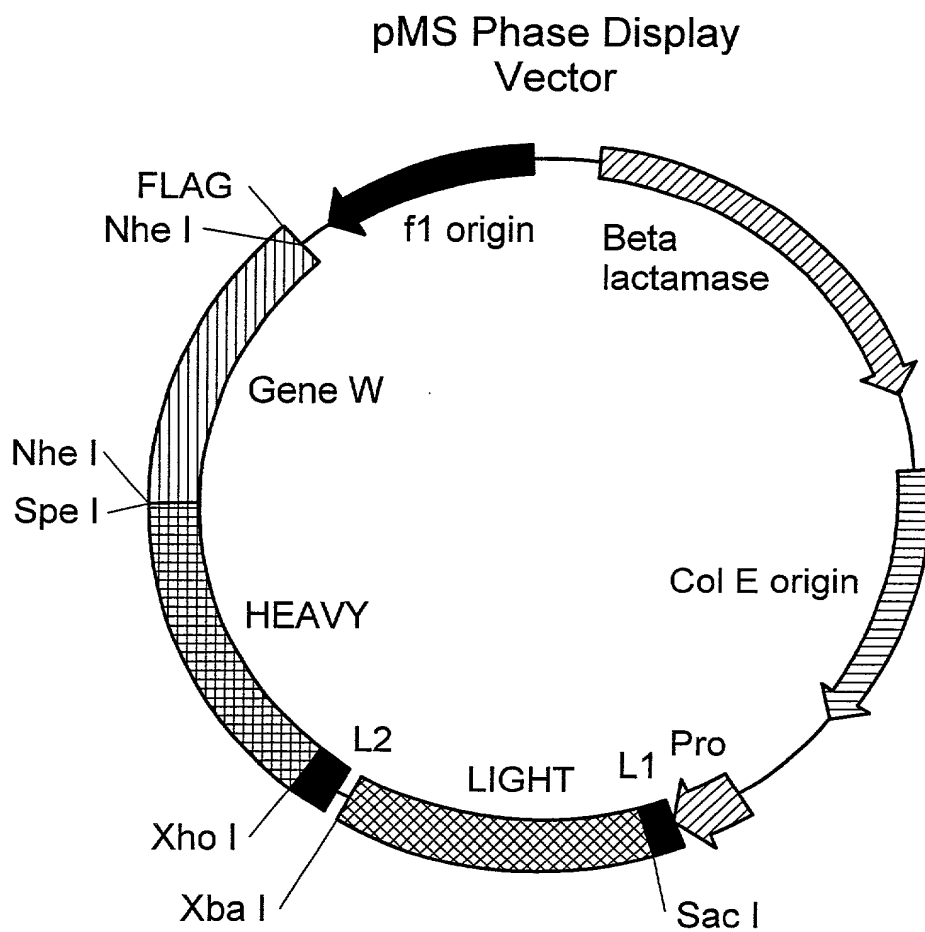
20. The method of claim 16 wherein said disease is graft-versus-host disease.

# ABSTRACT

The present invention relates to the identification of macaque antibodies to human B7.1 and B7.2 by screening of phage display libraries or monkey  
5 heterohybridomas obtained using B lymphocytes from B7.1 and/or B7.2 immunized monkeys. More specifically, the invention provides four monkey monoclonal antibodies 7B6, 16C10, 7C10 and 20C9 which inhibit the B7:CD28 pathway and thereby function as effective  
10 immunosuppressants. The invention further provides the complete DNA and amino acid sequences of the light and heavy chain of three primatized antibodies derived from those monkey monoclonal antibodies which bind B7.1 and possibly B7.2, primatized 7C10, primatized 7B6 and  
15 primatized 16C10. These primatized and monkey antibodies may be used as specific immunosuppressants, e.g., for the treatment of autoimmune diseases and to prevent organ transplant rejection.

669280" STEEBB

FIG. 1

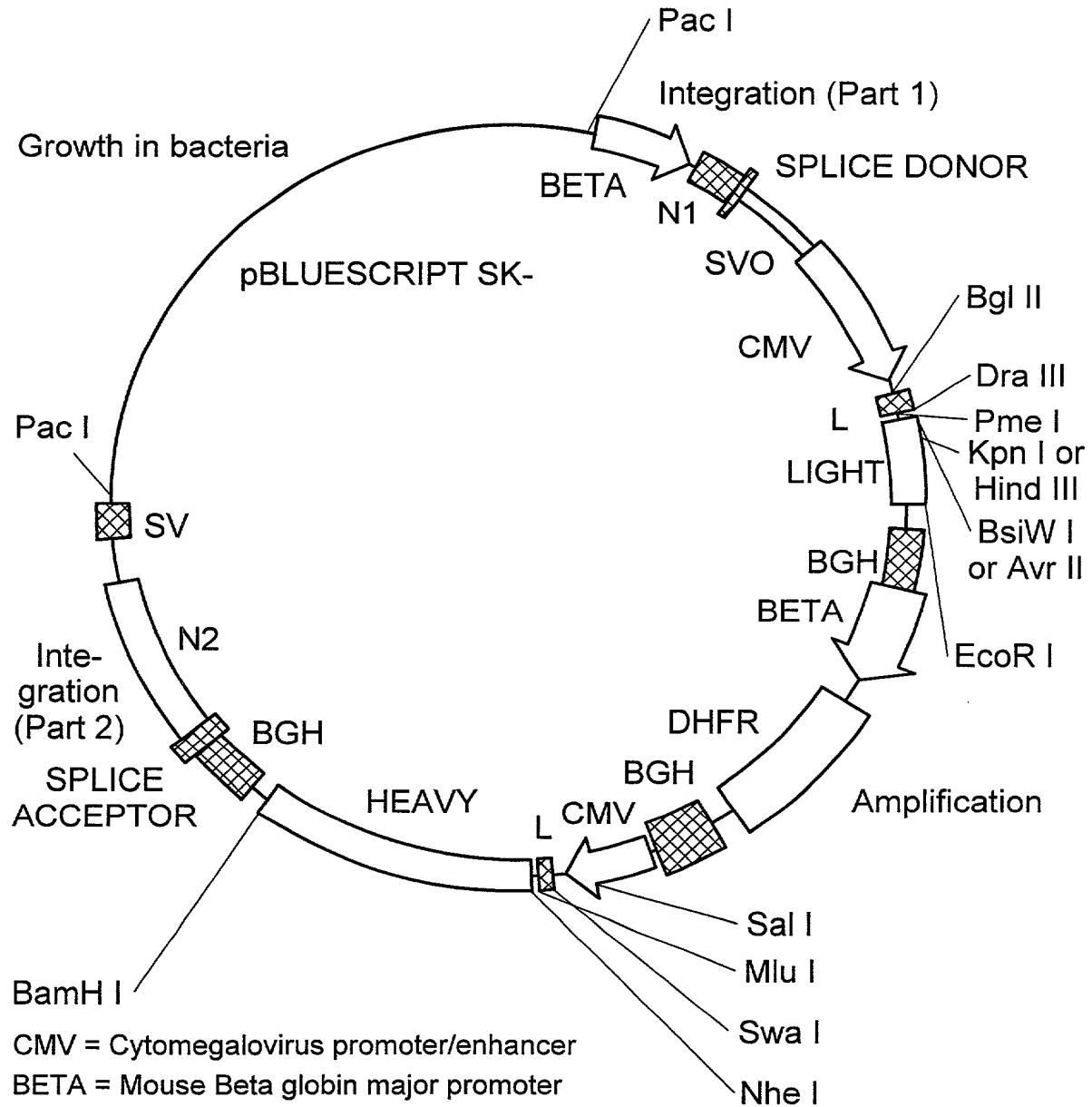


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# FIG. 2

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## NEOSPLA



CMV = Cytomegalovirus promoter/enhancer

BETA = Mouse Beta globin major promoter

SVO = SV40 origin

BGH = Bovine growth hormone polyadenylation

SV = SV40 polyadenylation

N1 = Neomycin phosphotransferase exon 1

N2 = Neomycin phosphotransferase exon 2

LIGHT = Human immunoglobulin kappa or lambda constant region

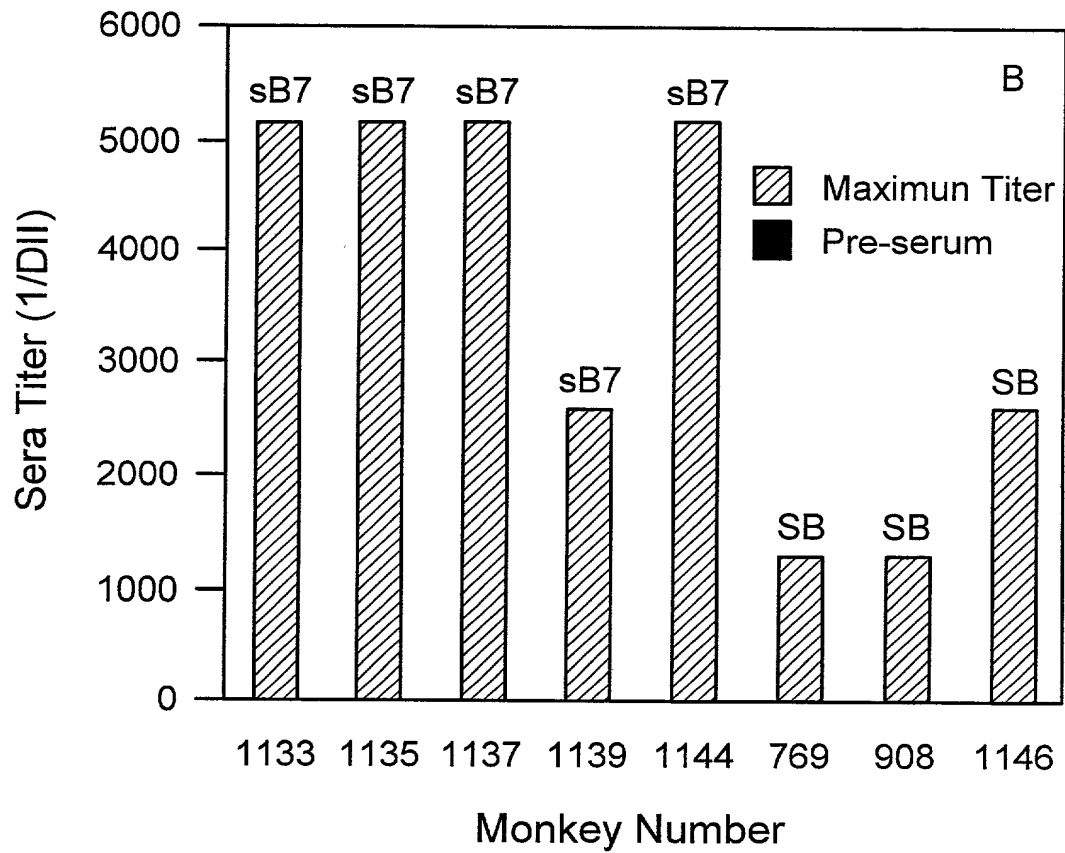
DHFR = Dihydrofolate Reductase

HEAVY = Human immunoglobulin gamma 1 or gamma 4 PE constant region

L = Leader

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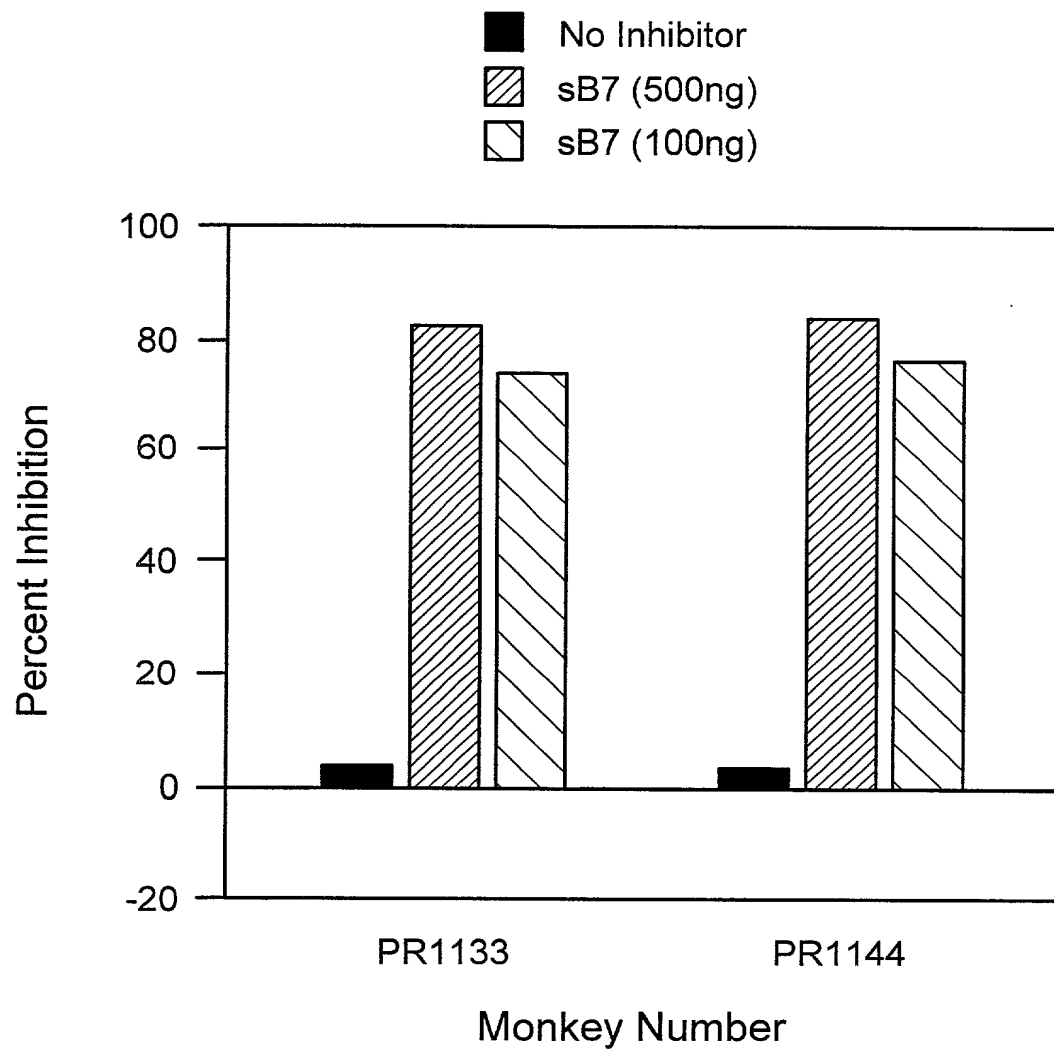
# FIG. 3



Monkey Serum Anti-B7.1 Titers Directed Against Cell Surface B7.1 on Transfected CHO Cells. Monkeys 1133-1139 were immunized with sB7.1. Monkeys 769-1146 were immunized with 50 million human B7 positive SB cells.

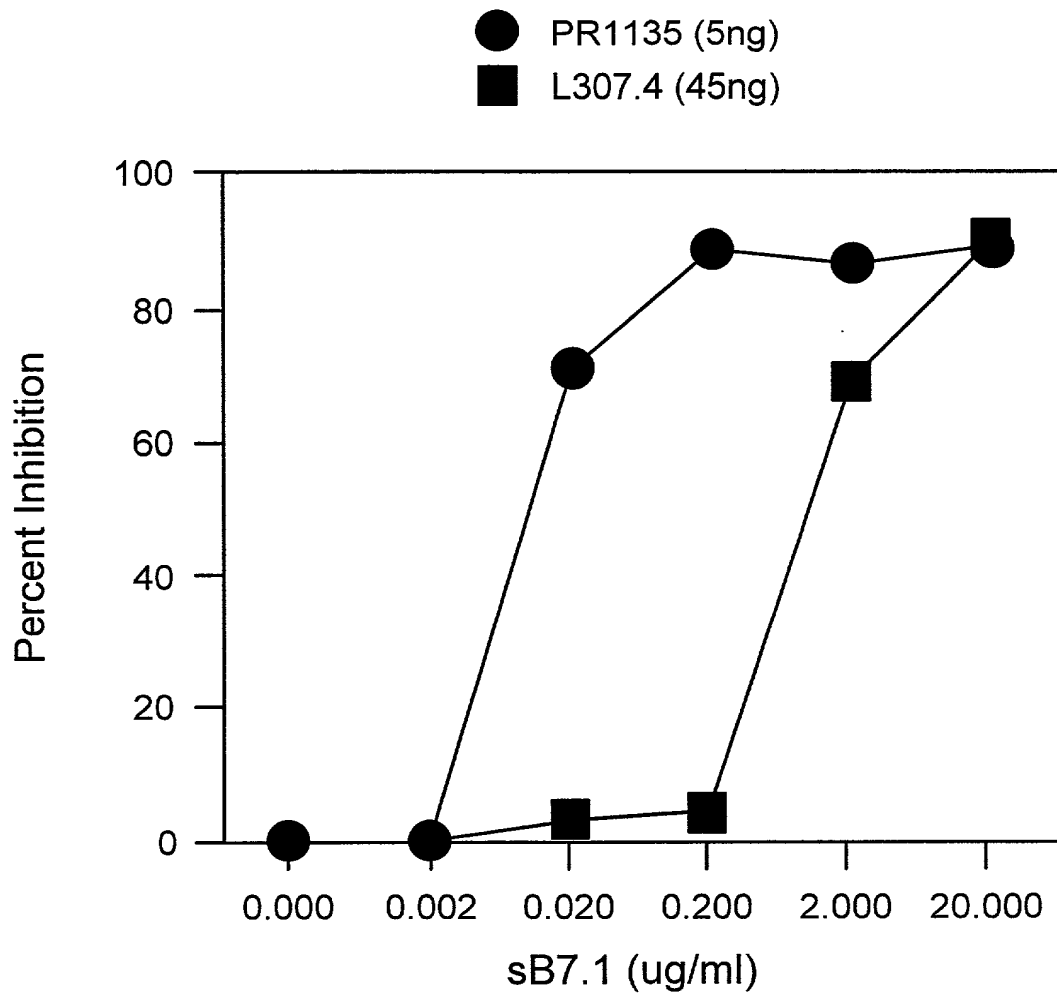


FIG. 4



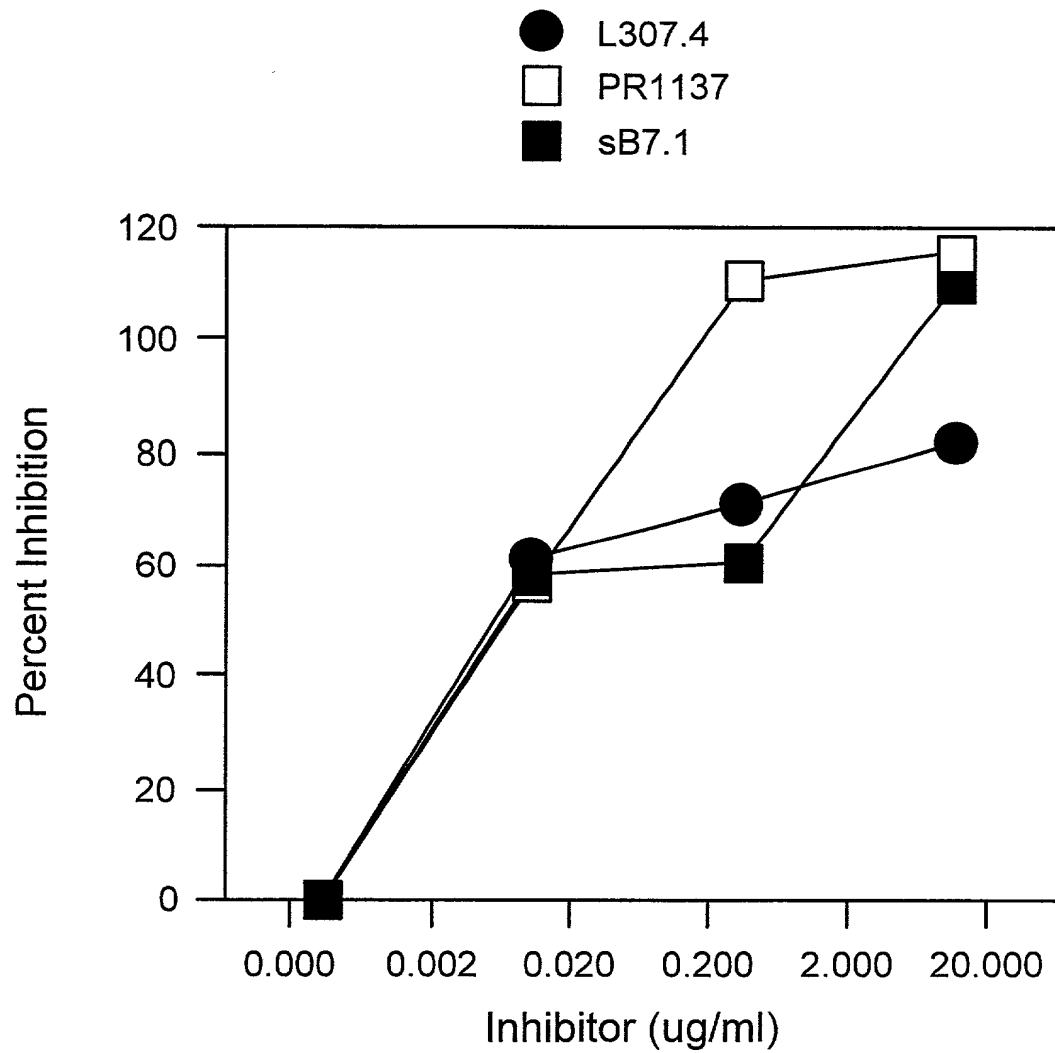
Inhibition of Radiolabeled sB7.1 Binding by sB7.1 Affinity-purified Monkey Antibodies in Presence of Unlabeled sB7 and MAb L307.4 Murine Anti-B7.1.

FIG. 5



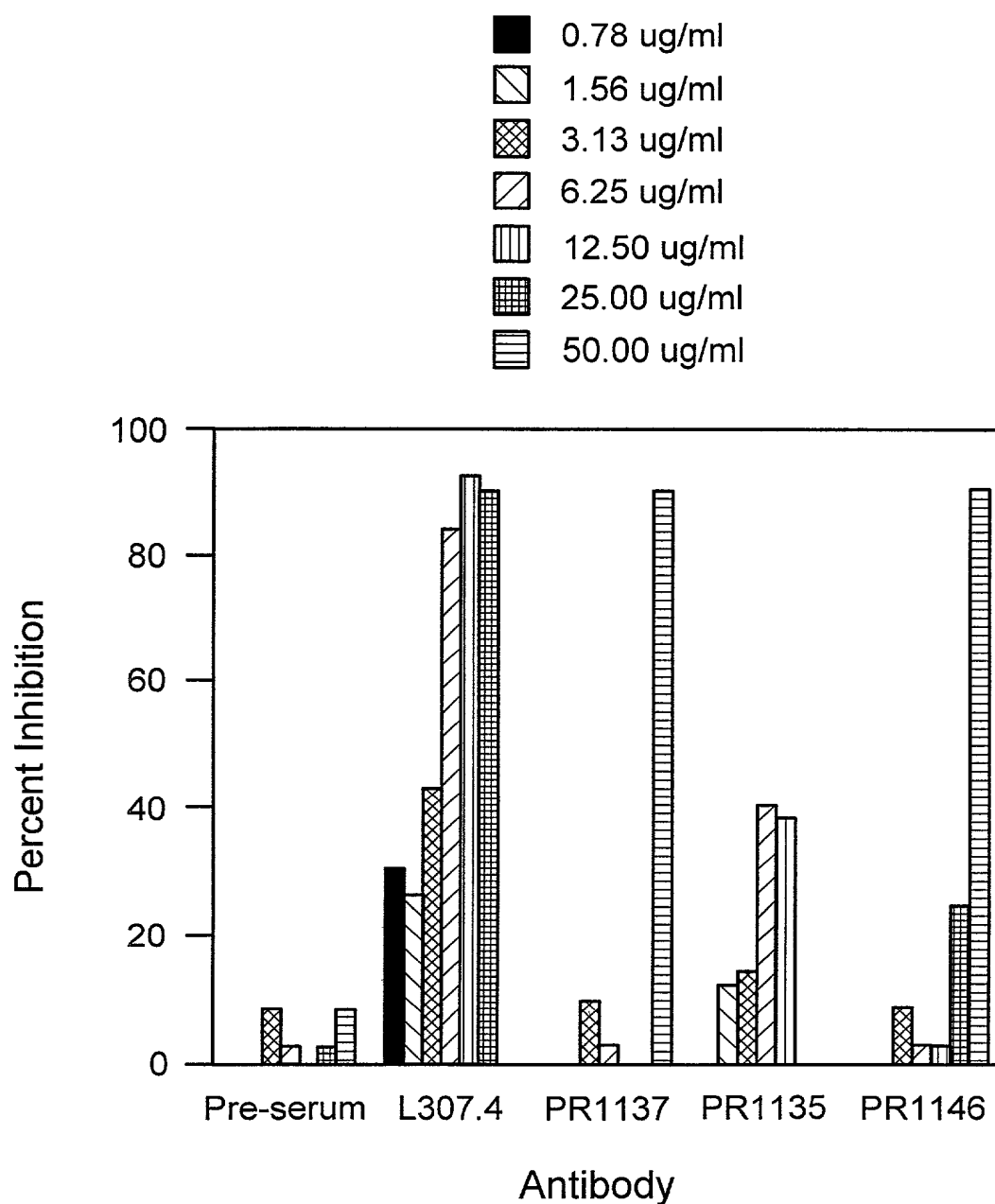
Inhibition of Binding of Radiolabeled Monkey 1135 and L307.4 Anti-B7.1 Antibodies to B7 Positive Human SB Cells by Competition With Affinity-Purified sB7.1.

FIG. 6



Inhibition of Radiolabeled B7-Ig Binding to Activated Human Peripheral Blood T Cells by Competing With Unlabeled sB7.1 Murine Anti-B7.1 (L307.4) and Monkey 1127 Affinity-purified Serum Antibodies.

# FIG. 7



Inhibition of IL-2 Production in Mixed Lymphocyte Cultures by Anti-B7.1 Affinity-purified Monkey Serum Antibodies. Assays at some concentrations for certain monkeys were not done, due to limiting amounts of purified antibody.

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 ACC GCC ACC CTG ACC ATC AAC GGG GTC GAG GCC GGG GAT GAG GCT  
 270 279 288 297 306

D Y Y C Q V W D R A S D H P V  
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 315 324 333 342 351

F G G G T R V T V L G Q P K A  
 TTC GGAGGAGGGACC CGGGTG ACC GTC CTA GGT CAG CCC AAG GCT  
 360 369 378 387 396

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 GCC CCC TCG GTC ACT CTG TTC CCG CCC TCC TCT GAG GAG CTT CAA  
 405 414 423 432 441

A N K A T L V C L I S D F Y P  
 GCC AAC AAG GCC ACA CTG GTG TGT CTC ATA AGT GAC TTC TAC CCG  
 450 459 468 477 486

FIG. 8A - 1

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 GGAGCC GTG ACA GTG GCC TGG AAG GCA GAT AGC AGC CCC GTC AAG  
 495 504 513 522 531

A G V E T T T P S K Q S N N K  
 GCGGGA GTG GAG ACC ACC ACA CCC TCC AAA CAA AGC AAC AAC AAG  
 540 549 558 567 576

Y A A S S Y L S L T P E Q W K  
 TAC GCGGCC AGC AGC TAC CTG AGC CTG ACG CCT GAG CAG TGG AAG  
 585 594 603 612 621

S H R S Y S C Q V T H E G S T  
 TCC CAC AGA AGC TAC AGC TGC CAG GTC ACG CAT GAA GGGAGC ACC  
 630 639 648 657 666

V E K T V A P T E C S  
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FIG. 8A - 2

669230-016960

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G  L  L  Q  P  S  E  T  L  S  R  T  C  V  V
GGA CTT CTG CAG CCT TCG GAG ACC CTG TCC CGC ACC TGC GTT GTC
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S  G  G  S  I  S  G  Y  Y  Y  W  T  W  I  R
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      135          144          153          162          171

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N  G  A  T  T  N  Y  N  P  S  L  K  S  R  V
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ACC ATT TCA AAA GAC ACG TCC AAG AAC CAG TTC TTC CTG AAC TTG
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N  S  V  T  D  A  D  T  A  V  Y  Y  C  A  R
AAT TCT GTG ACC GAC GCGGAC ACG GCC GTC TAT TAC TGT GCGAGA
      315          324          333          342          351

G  P  R  P  D  C  T  T  I  C  Y  G  G  W  V
GGCCCT CGC CCT GAT TGC ACA ACC ATT TGT TAT GGCGGCTGG GTC
      360          369          378          387          396

D  V  W  G  P  G  D  L  V  T  V  S  S  A  S
GAT GTC TGG GGCCCG GGA GAC CTG GTC ACC GTC TCC TCA GCT AGC
      405          414          423          432          441

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FIG. 8B - 1







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 450 459 468 477 486

FIG. 9A - 1

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 585 594 603 612 621

A D Y E K H K V Y A C E V T H  
 GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT  
 630 639 648 657 666

Q G L S S P V T K S F N R G E  
 CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG  
 675 684 693 702 711

C  
 TGT TGA  
 720

FIG. 9A - 2



K	S	T	S	G	G	T	A	A	L	G	C	L	V	K
AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG	GGC	TGC	CTG	GTC	AAG
495				504			513			522			531	
D	Y	F	P	E	P	V	T	V	S	W	N	S	G	A
GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA	GGC	GCC
540				549			558			567			576	
L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S
CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA
585				594			603			612			621	
G	L	Y	S	L	S	S	V	V	T	V	P	S	S	S
GGA	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC
630				639			648			657			666	
L	G	T	Q	T	Y	I	C	N	V	N	H	K	P	S
TTG	GGC	ACC	CAG	ACC	TAC	ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC
675				684			693			702			711	
N	T	K	V	D	K	K	A	E	P	K	S	C	D	K
AAC	ACC	AAG	GTG	GAC	AAG	AAA	GCA	GAG	CCC	AAA	TCT	TGT	GAC	AAA
720				729			738			747			756	
T	H	T	C	P	P	C	P	A	P	E	L	L	G	G
ACT	CAC	ACA	TGC	CCA	CCG	TGC	CCA	GCA	CCT	GAA	CTC	CTG	GGG	GGA
765				774			783			792			801	
P	S	V	F	L	F	P	P	K	P	K	D	T	L	M
CCG	TCA	GTC	TTC	CTC	TTC	CCC	CCA	AAA	CCC	AAG	GAC	ACC	CTC	ATG
810				819			828			837			846	
I	S	R	T	P	E	V	T	C	V	V	V	D	V	S
ATC	TCC	CGG	ACC	CCT	GAG	GTC	ACA	TGC	GTG	GTG	GTG	GAC	GTG	AGC
855				864			873			882			891	
H	E	D	P	E	V	K	F	N	W	Y	V	D	G	V
CAC	GAA	GAC	CCT	GAG	GTC	AAG	TTC	AAC	TGG	TAC	GTG	GAC	GGC	GTG
900				909			918			927			936	
E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N
GAG	GTG	CAT	AAT	GCC	AAG	ACA	AAG	CCG	CGG	GAG	GAG	CAG	TAC	AAC
945				954			963			972			981	
S	T	Y	R	V	V	S	V	L	T	V	L	H	Q	D
AGC	ACG	TAC	CGT	GTG	GTC	AGC	GTC	CTC	ACC	GTC	CTG	CAC	CAG	GAC
990				999			1008			1017			1026	

FIG. 9B - 2

W L N G K E Y K C K V S N K A  
TGG CTG AAT GGCAAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC  
1035 1044 1053 1062 1071

L P A P I E K T I S K A K G Q  
CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGGCAG  
1080 1089 1098 1107 1116

P R E P Q V Y T L P P S R D E  
CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG  
1125 1134 1143 1152 1161

L T K N Q V S L T C L V K G F  
CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGCTTC  
1170 1179 1188 1197 1206

Y P S D I A V E W E S N G Q P  
TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGGCAG CCG  
1215 1224 1233 1242 1251

E N N Y K T T P P V L D S D G  
GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC  
1260 1269 1278 1287 1296

S F F L Y S K L T V D K S R W  
TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG  
1305 1314 1323 1332 1341

Q Q G N V F S C S V M H E A L  
CAG CAG GGGAAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG  
1350 1359 1368 1377 1386

H N H Y T Q K S L S L S P G K  
CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA  
1395 1404 1413 1422 1431

TGA

FIG. 9B - 3

LENGTH OF 16C10 LAMBDA/PRIMATIZED: 711 bp; LISTED FROM: 1 TO: 711;  
 TRANSLATED FROM: 1 TO: 709 (ENTIRE REGION);  
 GENETIC CODE USED: UNIVERSAL; FRI, MAY 26, 1995 11:08 AM

```

FRAME 1 M  R  V  P  A  Q  L  L  G  L  L  L  L
          ATG AGG GTC CCC GCT CAG CTC CTG GGGCTC CTG CTG CTC
                9                18                27                36

W  L  P  G  A  R  C  E  S  V  L  T  Q  P  P
TGG CTC CCA GGT GCA CGA TGT GAG TCT GTC CTG ACA CAG CCG CCC
    45                54                63                72                81

S  V  S  G  A  P  G  Q  K  V  T  I  S  C  T
TCA GTG TCT GGGGCC CCA GGGCAG AAG GTC ACC ATC TCG TGC ACT
    90                99                108                117                126

G  S  T  S  N  I  G  G  Y  D  L  H  W  Y  Q
GGGAGC ACC TCC AAC ATT GGA GGT TAT GAT CTA CAT TGG TAC CAG
    135                144                153                162                171

Q  L  P  G  T  A  P  K  L  L  I  Y  D  I  N
CAG CTC CCA GGAACG GCC CCC AAA CTC CTC ATC TAT GAC ATT AAC
    180                189                198                207                216

K  R  P  S  G  I  S  D  R  F  S  G  S  K  S
AAG CGA CCC TCA GGAATT TCT GAC CGA TTC TCT GGCTCC AAG TCT
    225                234                243                252                261

G  T  A  A  S  L  A  I  T  G  L  Q  T  E  D
GGT ACC GCGGCC TCC CTG GCC ATC ACT GGGCTC CAG ACT GAG GAT
    270                279                288                297                306

E  A  D  Y  Y  C  Q  S  Y  D  S  S  L  N  A
GAG GCT GAT TAT TAC TGC CAG TCC TAT GAC AGC AGC CTG AAT GCT
    315                324                333                342                351

Q  V  F  G  G  G  T  R  L  T  V  L  G  Q  P
CAG GTA TTC GGAGGA GGGACC CGGCTG ACC GTC CTA GGT CAG CCC
    360                369                378                387                396

K  A  A  P  S  V  T  L  F  P  P  S  S  E  E
AAG GCT GCC CCC TCG GTC ACT CTG TTC CCG CCC TCC TCT GAG GAG
    405                414                423                432                441

L  Q  A  N  K  A  T  L  V  C  L  I  S  D  F
CTT CAA GCC AAC AAG GCC ACA CTG GTG TGT CTC ATA AGT GAC TTC
    450                459                468                477                486

```

FIG. 10A - 1





LENGTH OF 16C10 HEAVY/PRIMATIZED: 1431 bp; LISTED FROM: 1 TO: 1431;  
 TRANSLATED FROM: 1 TO: 1429 (ENTIRE REGION);  
 GENETIC CODE USED: UNIVERSAL; FRI, MAY 26, 1995 11:08 AM

FRAME 1 M K H L W F F L L L V A A  
 ATG AAA CAC CTG TGG TTC TTC CTC CTC CTG GTG GCA GCT  
 9 18 27 36

P R W V L S Q V Q L Q E S G P  
 CCC AGA TGG GTC CTG TCC CAG GTG CAG CTG CAG GAG TCG GGCCCA  
 45 54 63 72 81

G L V K P S E T L S L T C A V  
 GGA CTG GTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GCT GTC  
 90 99 108 117 126

S G G S I S G G Y G W G W I R  
 TCT GGT GGCTCC ATC AGC GGT GGT TAT GGCTGG GGCTGG ATC CGC  
 135 144 153 162 171

Q P P G K G L E W I G S F Y S  
 CAG CCC CCA GGGAAG GGGCTG GAG TGG ATT GGGAGT TTC TAT AGT  
 180 189 198 207 216

S S G N T Y Y N P S L K S Q V  
 AGT AGT GGGAAC ACC TAC TAC AAC CCC TCC CTC AAG AGT CAA GTC  
 225 234 243 252 261

T I S T D T S K N Q F S L K L  
 ACC ATT TCA ACA GAC ACG TCC AAG AAC CAG TTC TCC CTG AAG CTG  
 270 279 288 297 306

N S M T A A D T A V Y Y C V R  
 AAC TCT ATG ACC GCC GCG GAC ACG GCC GTG TAT TAC TGT GTG AGA  
 315 324 333 342 351

D R L F S V V G M V Y N N W F  
 GAT CGT CTT TTT TCA GTT GTT GGAATG GTT TAC AAC AAC TGG TTC  
 360 369 378 387 396

D V W G P G V L V T V S S A S  
 GAT GTC TGG GGC CCG GGA GTC CTG GTC ACC GTC TCC TCA GCT AGC  
 405 414 423 432 441

T K G P S V F P L A P S S K S  
 ACC AAG GGCCCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC  
 450 459 468 477 486

FIG. 10B - 1



N G K E Y K C K V S N K A L P  
 AAT GGCAAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA  
 1035 1044 1053 1062 1071

A P I E K T I S K A K G Q P R  
 GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GCC AAA CCC CGA  
 1080 1089 1098 1107 1116

E P Q V Y T L P P S R D E L T  
 GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC  
 1125 1134 1143 1152 1161

K N Q V S L T C L V K G F Y P  
 AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGCTTC TAT CCC  
 1170 1179 1188 1197 1206

S D I A V E W E S N G Q P E N  
 AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC  
 1215 1224 1233 1242 1251

N Y K T T P P V L D S D G S F  
 AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC TCC TCC TTC  
 1260 1269 1278 1287 1296

F L Y S K L T V D K S R W Q Q  
 TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG  
 1305 1314 1323 1332 1341

G N V F S C S V M H E A L H N  
 GGGAAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC  
 1350 1359 1368 1377 1386

H Y T Q K S L S L S P G K  
 CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA  
 1395 1404 1413 1422 1431

FIG. 10B - 3

**COMBINED DECLARATION AND POWER OF ATTORNEY  
FOR UTILITY PATENT APPLICATION**

Attorney's Docket No.

012712-131

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I BELIEVE I AM THE ORIGINAL, FIRST AND SOLE INVENTOR (if only one name is listed below) OR AN ORIGINAL, FIRST AND JOINT INVENTOR (if more than one name is listed below) OF THE SUBJECT MATTER WHICH IS CLAIMED AND FOR WHICH A PATENT IS SOUGHT ON THE INVENTION ENTITLED:

MONKEY MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN B7.1 AND/OR B7.2 PRIMATIZED FORMS

THEREOF, PHARMACEUTICAL COMPOSITIONS CONTAINING, AND USE THEREOF AS

IMMUNOSUPPRESSANTS

the specification of which

(check one)

☐

is attached hereto;

☒

was filed on June 7, 1995 as

Application No. 08/487,550

and was amended on \_\_\_\_\_;  
(if applicable)

I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE;

I ACKNOWLEDGE THE DUTY TO DISCLOSE TO THE OFFICE ALL INFORMATION KNOWN TO ME TO BE MATERIAL TO PATENTABILITY AS DEFINED IN TITLE 37, CODE OF FEDERAL REGULATIONS, Sec. 1.56 (as amended effective March 16, 1992);

I do not know and do not believe the said invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to said application; that said invention was not in public use or on sale in the United States of America more than one year prior to said application; that said invention has not been patented or made the subject of an inventor's certificate issued before the date of said application in any country foreign to the United States of America on any application filed by me or my legal representatives or assigns more than twelve months prior to said application;

I hereby claim foreign priority benefits under Title 35, United States Code Sec. 119 and/or Sec. 365 of any foreign application(s) for patent or inventor's certificate as indicated below and have also identified below any foreign application for patent or inventor's certificate on this invention having a filing date before that of the application(s) on which priority is claimed:

# COMBINED DECLARATION AND POWER OF ATTORNEY

Attorney's Docket No.

012712-131

COUNTRY/INTERNATIONAL

APPLICATION NUMBER

DATE OF FILING  
(day, month, year)

PRIORITY  
CLAIMED

YES\_ NO\_

YES\_ NO\_

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis	17,337	Ralph L. Freeland, Jr.	16,110	William C. Rowland	30,888
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Joseph R. Magnone	24,239	R. Danny Huntington	27,903	William H. Benz	25,952
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Frederick G. Michaud, Jr.	26,003	Teresa Stanek Rea	30,427	Matthew L. Schneider	32,814
Alan E. Kopecki	25,813	Robert E. Krebs	25,885	Michael G. Savage	32,596
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR

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SIGNATURE

*Darrell R. Anderson*

DATE

12-18-97

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Nabil HANNA

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*Nabil Hanna*

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CITIZENSHIP

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003346 032393

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POST OFFICE ADDRESS 1155 Thomas Avenue, San Diego, CA 92109				
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY Cheryl HEARD		SIGNATURE <i>Cheryl Heard</i>		DATE 12-18-97
RESIDENCE 1225 Via Montoro, Encinitas, CA 92024		CITIZENSHIP USA		
POST OFFICE ADDRESS 1225 Via Montoro, Encinitas, CA 92024				
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE
RESIDENCE		CITIZENSHIP		
POST OFFICE ADDRESS				
FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE
RESIDENCE		CITIZENSHIP		
POST OFFICE ADDRESS				
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE
RESIDENCE		CITIZENSHIP		
POST OFFICE ADDRESS				
FULL NAME OF NINTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE
RESIDENCE		CITIZENSHIP		
POST OFFICE ADDRESS				
FULL NAME OF TENTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE
RESIDENCE		CITIZENSHIP		
POST OFFICE ADDRESS				
FULL NAME OF ELEVENTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE
RESIDENCE		CITIZENSHIP		
POST OFFICE ADDRESS				
FULL NAME OF TWELFTH JOINT INVENTOR, IF ANY		SIGNATURE		DATE
RESIDENCE		CITIZENSHIP		
POST OFFICE ADDRESS				

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Anderson, Darrell R.
- (ii) TITLE OF INVENTION: "MONKEY MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN B7.1 AND/OR B7.2 PRIMATIZED FORMS THEREOF, PHARMACEUTIAL COMPOSITIONS CONTAINING, AND USE THEREOF AS IMMUNOSUPPRESSANTS"
- (iii) NUMBER OF SEQUENCES: 12
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: BURNS, DOANE, SWECKER & MATHIS
  - (B) STREET: 699 Prince Street
  - (C) CITY: Alexandria
  - (D) STATE: VA
  - (E) COUNTRY: USA
  - (F) ZIP: 22314
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER: US 08/487,550
  - (B) FILING DATE: 07-JUN-1995
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Teskin, Robin L.
  - (B) REGISTRATION NUMBER: 35,030
  - (C) REFERENCE/DOCKET NUMBER: 012712-131
- (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: 703 836 6620
  - (B) TELEFAX: 703 836 2021

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 705 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: not relevant
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 1..705

## (ix) FEATURE:

(A) NAME/KEY: mat\_peptide  
(B) LOCATION: 1..705

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG	AGG	GTC	CCC	GCT	CAG	CTC	CTG	GGG	CTC	CTG	CTG	CTC	TGG	CTC	CCA	48
Met	Arg	Val	Pro	Ala	Gln	Leu	Leu	Gly	Leu	Leu	Leu	Leu	Trp	Leu	Pro	
1				5				10					15			
GGT	GCA	CGA	TGT	GCC	TAT	GAA	CTG	ACT	CAG	CCA	CCC	TCG	GTG	TCA	GTG	96
Gly	Ala	Arg	Cys	Ala	Tyr	Glu	Leu	Thr	Gln	Pro	Pro	Ser	Val	Ser	Val	
			20				25						30			
TCC	CCA	GGA	CAG	ACG	GCC	AGG	ATC	ACC	TGT	GGG	GGA	GAC	AAC	AGT	AGA	144
Ser	Pro	Gly	Gln	Thr	Ala	Arg	Ile	Thr	Cys	Gly	Gly	Asp	Asn	Ser	Arg	
			35				40					45				
AAT	GAA	TAT	GTC	CAC	TGG	TAC	CAG	CAG	AAG	CCA	GCG	CGG	GCC	CCT	ATA	192
Asn	Glu	Tyr	Val	His	Trp	Tyr	Gln	Gln	Lys	Pro	Ala	Arg	Ala	Pro	Ile	
	50					55				60						
CTG	GTC	ATC	TAT	GAT	GAT	AGT	GAC	CGG	CCC	TCA	GGG	ATC	CCT	GAG	CGA	240
Leu	Val	Ile	Tyr	Asp	Asp	Ser	Asp	Arg	Pro	Ser	Gly	Ile	Pro	Glu	Arg	
	65				70				75					80		
TTC	TCT	GGC	TCC	AAA	TCA	GGG	AAC	ACC	GCC	ACC	CTG	ACC	ATC	AAC	GGG	288
Phe	Ser	Gly	Ser	Lys	Ser	Gly	Asn	Thr	Ala	Thr	Leu	Thr	Ile	Asn	Gly	
				85				90						95		
GTC	GAG	GCC	GGG	GAT	GAG	GCT	GAC	TAT	TAC	TGT	CAG	GTG	TGG	GAC	AGG	336
Val	Glu	Ala	Gly	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Val	Trp	Asp	Arg	
			100				105					110				
GCT	AGT	GAT	CAT	CCG	GTC	TTC	GGA	GGA	GGG	ACC	CGG	GTG	ACC	GTC	CTA	384
Ala	Ser	Asp	His	Pro	Val	Phe	Gly	Gly	Gly	Thr	Arg	Val	Thr	Val	Leu	
		115					120					125				
GGT	CAG	CCC	AAG	GCT	GCC	CCC	TCG	GTC	ACT	CTG	TTC	CCG	CCC	TCC	TCT	432
Gly	Gln	Pro	Lys	Ala	Ala	Pro	Ser	Val	Thr	Leu	Phe	Pro	Pro	Ser	Ser	
	130					135					140					
GAG	GAG	CTT	CAA	GCC	AAC	AAG	GCC	ACA	CTG	GTG	TGT	CTC	ATA	AGT	GAC	480
Glu	Glu	Leu	Gln	Ala	Asn	Lys	Ala	Thr	Leu	Val	Cys	Leu	Ile	Ser	Asp	
	145				150				155						160	
TTC	TAC	CCG	GGA	GCC	GTG	ACA	GTG	GCC	TGG	AAG	GCA	GAT	AGC	AGC	CCC	528
Phe	Tyr	Pro	Gly	Ala	Val	Thr	Val	Ala	Trp	Lys	Ala	Asp	Ser	Ser	Pro	
			165					170					175			
GTC	AAG	GCG	GGA	GTG	GAG	ACC	ACC	ACA	CCC	TCC	AAA	CAA	AGC	AAC	AAC	576
Val	Lys	Ala	Gly	Val	Glu	Thr	Thr	Thr	Pro	Ser	Lys	Gln	Ser	Asn	Asn	

09333916-082699



180										185					190					
AAG	TAC	GCG	GCC	AGC	AGC	TAC	CTG	AGC	CTG	ACG	CCT	GAG	CAG	TGG	AAG	624				
Lys	Tyr	Ala	Ala	Ser	Ser	Tyr	Leu	Ser	Leu	Thr	Pro	Glu	Gln	Trp	Lys					
		195					200					205								
TCC	CAC	AGA	AGC	TAC	AGC	TGC	CAG	GTC	ACG	CAT	GAA	GGG	AGC	ACC	GTG	672				
Ser	His	Arg	Ser	Tyr	Ser	Cys	Gln	Val	Thr	His	Glu	Gly	Ser	Thr	Val					
	210					215					220									
GAG	AAG	ACA	GTG	GCC	CCT	ACA	GAA	TGT	TCA	TGA						705				
Glu	Lys	Thr	Val	Ala	Pro	Thr	Glu	Cys	Ser	*										
225					230					235										

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 235 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Arg	Val	Pro	Ala	Gln	Leu	Leu	Gly	Leu	Leu	Leu	Leu	Trp	Leu	Pro	
1				5					10					15		
Gly	Ala	Arg	Cys	Ala	Tyr	Glu	Leu	Thr	Gln	Pro	Pro	Ser	Val	Ser	Val	
			20					25					30			
Ser	Pro	Gly	Gln	Thr	Ala	Arg	Ile	Thr	Cys	Gly	Gly	Asp	Asn	Ser	Arg	
		35					40					45				
Asn	Glu	Tyr	Val	His	Trp	Tyr	Gln	Gln	Lys	Pro	Ala	Arg	Ala	Pro	Ile	
	50					55					60					
Leu	Val	Ile	Tyr	Asp	Asp	Ser	Asp	Arg	Pro	Ser	Gly	Ile	Pro	Glu	Arg	
65					70					75				80		
Phe	Ser	Gly	Ser	Lys	Ser	Gly	Asn	Thr	Ala	Thr	Leu	Thr	Ile	Asn	Gly	
				85					90					95		
Val	Glu	Ala	Gly	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Val	Trp	Asp	Arg	
			100					105					110			
Ala	Ser	Asp	His	Pro	Val	Phe	Gly	Gly	Gly	Thr	Arg	Val	Thr	Val	Leu	
		115					120					125				
Gly	Gln	Pro	Lys	Ala	Ala	Pro	Ser	Val	Thr	Leu	Phe	Pro	Pro	Ser	Ser	
	130					135					140					
Glu	Glu	Leu	Gln	Ala	Asn	Lys	Ala	Thr	Leu	Val	Cys	Leu	Ile	Ser	Asp	
145					150					155					160	

665230" 9T6EE60

(2) INFORMATION FOR SEQ ID NO:3:

(A) LENGTH: 1431 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: not relevant  
(D) TOPOLOGY: linear

(ix) FEATURE:

(ix) FEATURE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG Met 1	AAA Lys	CAC His	CTG Leu	TGG Trp 5	TTC Phe	TTC Phe	CTC Leu	CTC Leu	CTG Leu 10	GTG Val	GCA Ala	GCT Ala	CCC Pro	AGA Arg 15	TGG Trp	48
GTC Val	CTG Leu	TCC Ser	CAG Gln 20	GTG Val	AAG Lys	CTG Leu	CAG Gln	CAG Gln 25	TGG Trp	GGC Gly	GAA Glu	GGA Gly	CTT Leu 30	CTG Leu	CAG Gln	96
CCT Pro	TCG Ser	GAG Glu 35	ACC Thr	CTG Leu	TCC Ser	CGC Arg	ACC Thr 40	TGC Cys	GTT Val	GTC Val	TCT Ser	GGT Gly 45	GGC Gly	TCC Ser	ATC Ile	144
AGC Ser 50	GGT Gly	TAC Tyr	TAC Tyr	TAC Tyr	TGG Trp 55	ACC Thr	TGG Trp	ATC Ile	CGC Arg	CAG Gln	ACC Thr 60	CCA Pro	GGG Gly	AGG Arg	GGA Gly	192
CTG Leu 65	GAG Glu	TGG Trp	ATT Ile	GGC Gly 70	CAT His	ATT Ile	TAT Tyr	GGT Gly	AAT Asn 75	GGT Gly	GCG Ala	ACC Thr	ACC Thr	AAC Asn	TAC Tyr 80	240

AAT	CCC	TCC	CTC	AAG	AGT	CGA	GTC	ACC	ATT	TCA	AAA	GAC	ACG	TCC	AAG	288
Asn	Pro	Ser	Leu	Lys	Ser	Arg	Val	Thr	Ile	Ser	Lys	Asp	Thr	Ser	Lys	
				85					90					95		
AAC	CAG	TTC	TTC	CTG	AAC	TTG	AAT	TCT	GTG	ACC	GAC	GCG	GAC	ACG	GCC	336
Asn	Gln	Phe	Phe	Leu	Asn	Leu	Asn	Ser	Val	Thr	Asp	Ala	Asp	Thr	Ala	
			100					105					110			
GTC	TAT	TAC	TGT	GCG	AGA	GGC	CCT	CGC	CCT	GAT	TGC	ACA	ACC	ATT	TGT	384
Val	Tyr	Tyr	Cys	Ala	Arg	Gly	Pro	Arg	Pro	Asp	Cys	Thr	Thr	Ile	Cys	
		115					120					125				
TAT	GGC	GGC	TGG	GTC	GAT	GTC	TGG	GGC	CCG	GGA	GAC	CTG	GTC	ACC	GTC	432
Tyr	Gly	Gly	Trp	Val	Asp	Val	Trp	Gly	Pro	Gly	Asp	Leu	Val	Thr	Val	
	130					135					140					
TCC	TCA	GCT	AGC	ACC	AAG	GGC	CCA	TCG	GTC	TTC	CCC	CTG	GCA	CCC	TCC	480
Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	
					150					155					160	
TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG	GGC	TGC	CTG	GTC	AAG	528
Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	
				165					170					175		
GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA	GGC	GCC	CTG	576
Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	
			180					185					190			
ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA	CTC	624
Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	
		195					200					205				
TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	672
Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	
	210					215					220					
CAG	ACC	TAC	ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG	720
Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	
	225				230					235					240	
GAC	AAG	AAA	GCA	GAG	CCC	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	CCA	768
Asp	Lys	Lys	Ala	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	
				245					250					255		
CCG	TGC	CCA	GCA	CCT	GAA	CTC	CTG	GGG	GGA	CCG	TCA	GTC	TTC	CTC	TTC	816
Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	
			260					265					270			
CCC	CCA	AAA	CCC	AAG	GAC	ACC	CTC	ATG	ATC	TCC	CGG	ACC	CCT	GAG	GTC	864
Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	
		275					280					285				
ACA	TGC	GTG	GTG	GTG	GAC	GTG	AGC	CAC	GAA	GAC	CCT	GAG	GTC	AAG	TTC	912
Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	
	290					295					300					

AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG 960  
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro  
305 310 315 320  
CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC 1008  
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr  
325 330 335  
GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC 1056  
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val  
340 345 350  
TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC 1104  
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala  
355 360 365  
AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG 1152  
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg  
370 375 380  
GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC 1200  
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly  
385 390 395 400  
TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG 1248  
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro  
405 410 415  
GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC 1296  
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser  
420 425 430  
TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG 1344  
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln  
435 440 445  
GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC 1392  
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His  
450 455 460  
TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA 1431  
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys \*  
465 470 475

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 477 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met 1	Lys	His	Leu	Trp 5	Phe	Phe	Leu	Leu	Leu 10	Val	Ala	Ala	Pro	Arg 15	Trp
Val	Leu	Ser	Gln 20	Val	Lys	Leu	Gln	Gln 25	Trp	Gly	Glu	Gly	Leu 30	Leu	Gln
Pro	Ser	Glu 35	Thr	Leu	Ser	Arg	Thr 40	Cys	Val	Val	Ser	Gly 45	Gly	Ser	Ile
Ser	Gly 50	Tyr	Tyr	Tyr	Trp	Thr 55	Trp	Ile	Arg	Gln	Thr 60	Pro	Gly	Arg	Gly
Leu 65	Glu	Trp	Ile	Gly	His 70	Ile	Tyr	Gly	Asn	Gly 75	Ala	Thr	Thr	Asn	Tyr 80
Asn	Pro	Ser	Leu	Lys 85	Ser	Arg	Val	Thr	Ile 90	Ser	Lys	Asp	Thr	Ser 95	Lys
Asn	Gln	Phe	Phe 100	Leu	Asn	Leu	Asn	Ser 105	Val	Thr	Asp	Ala	Asp 110	Thr	Ala
Val	Tyr	Tyr 115	Cys	Ala	Arg	Gly	Pro 120	Arg	Pro	Asp	Cys	Thr 125	Thr	Ile	Cys
Tyr	Gly 130	Gly	Trp	Val	Asp	Val 135	Trp	Gly	Pro	Gly	Asp 140	Leu	Val	Thr	Val
Ser 145	Ser	Ala	Ser	Thr	Lys 150	Gly	Pro	Ser	Val	Phe 155	Pro	Leu	Ala	Pro	Ser 160
Ser	Lys	Ser	Thr	Ser 165	Gly	Gly	Thr	Ala	Ala 170	Leu	Gly	Cys	Leu	Val 175	Lys
Asp	Tyr	Phe	Pro 180	Glu	Pro	Val	Thr	Val 185	Ser	Trp	Asn	Ser	Gly 190	Ala	Leu
Thr	Ser	Gly 195	Val	His	Thr	Phe	Pro 200	Ala	Val	Leu	Gln	Ser 205	Ser	Gly	Leu
Tyr	Ser 210	Leu	Ser	Ser	Val	Val 215	Thr	Val	Pro	Ser	Ser 220	Ser	Leu	Gly	Thr
Gln 225	Thr	Tyr	Ile	Cys	Asn 230	Val	Asn	His	Lys	Pro 235	Ser	Asn	Thr	Lys	Val 240
Asp	Lys	Lys	Ala	Glu 245	Pro	Lys	Ser	Cys	Asp 250	Lys	Thr	His	Thr	Cys 255	Pro
Pro	Cys	Pro	Ala 260	Pro	Glu	Leu	Leu	Gly 265	Gly	Pro	Ser	Val	Phe 270	Leu	Phe
Pro	Pro	Lys 275	Pro	Lys	Asp	Thr	Leu 280	Met	Ile	Ser	Arg	Thr 285	Pro	Glu	Val
Thr	Cys 290	Val	Val	Val	Asp	Val 295	Ser	His	Glu	Asp	Pro 300	Glu	Val	Lys	Phe

Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro
305					310					315					320
Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr
				325					330					335	
Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val
			340					345					350		
Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala
		355					360					365			
Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg
	370					375					380				
Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly
385					390					395					400
Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro
				405					410					415	
Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser
			420					425					430		
Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln
		435					440					445			
Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His
	450					455					460				
Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	*			
465					470					475					

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 720 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: not relevant  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 1..720

(ix) **FEATURE:**

- ```
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..720
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATG AGC CTC CCT GCT CAG CTC CTC GGG CTG CTA TTG CTC TGC GTC CCC

|                   |                  |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |     |
|-------------------|------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-----|
| Met<br>1          | Ser              | Leu               | Pro               | Ala<br>5          | Gln               | Leu               | Leu               | Gly               | Leu<br>10         | Leu               | Leu               | Leu               | Cys               | Val<br>15         | Pro               |     |
| GGG<br>Gly        | TCC<br>Ser       | AGT<br>Ser        | GGG<br>Gly<br>20  | GAA<br>Glu        | GTT<br>Val        | GTG<br>Val        | ATG<br>Met        | ACT<br>Thr<br>25  | CAG<br>Gln        | TCT<br>Ser        | CCA<br>Pro        | CTG<br>Leu        | TCC<br>Ser<br>30  | CTT<br>Leu        | CCC<br>Pro        | 96  |
| ATC<br>Ile        | ACA<br>Thr       | CCT<br>Pro<br>35  | GGA<br>Gly        | GAG<br>Glu        | CCG<br>Pro        | GCC<br>Ala        | TCC<br>Ser<br>40  | ATC<br>Ile        | TCC<br>Ser        | TGT<br>Cys        | AGG<br>Arg        | TCT<br>Ser<br>45  | AGT<br>Ser        | CAA<br>Gln        | AGC<br>Ser        | 144 |
| CTT<br>Leu        | AAA<br>Lys<br>50 | CAC<br>His        | AGT<br>Ser        | AAT<br>Asn        | GGA<br>Gly        | GAC<br>Asp<br>55  | ACC<br>Thr        | TTC<br>Phe        | CTG<br>Leu        | AGT<br>Ser        | TGG<br>Trp<br>60  | TAT<br>Tyr        | CAG<br>Gln        | CAG<br>Gln        | AAG<br>Lys        | 192 |
| CCA<br>Pro<br>65  | GGC<br>Gly       | CAA<br>Gln        | CCT<br>Pro        | CCA<br>Pro        | AGG<br>Arg<br>70  | CTC<br>Leu        | CTG<br>Leu        | ATT<br>Ile        | TAT<br>Tyr        | AAG<br>Lys<br>75  | GTT<br>Val        | TCT<br>Ser        | AAC<br>Asn        | CGG<br>Arg        | GAC<br>Asp<br>80  | 240 |
| TCT<br>Ser        | GGG<br>Gly       | GTC<br>Val        | CCA<br>Pro        | GAC<br>Asp<br>85  | AGA<br>Arg        | TTC<br>Phe        | AGC<br>Ser        | GGC<br>Gly<br>90  | AGT<br>Ser        | GGG<br>Gly        | GCA<br>Ala        | GGG<br>Gly        | ACA<br>Thr        | GAT<br>Asp<br>95  | TTC<br>Phe        | 288 |
| ACA<br>Thr        | CTG<br>Leu       | AAA<br>Lys<br>100 | ATC<br>Ile        | AGC<br>Ser        | GCA<br>Ala        | GTG<br>Val        | GAG<br>Glu        | GCT<br>Ala<br>105 | GAA<br>Glu        | GAT<br>Asp        | GTT<br>Val        | GGG<br>Gly<br>110 | GTT<br>Val        | TAT<br>Tyr        | TTC<br>Phe        | 336 |
| TGC<br>Cys        | GGG<br>Gly       | CAA<br>Gln<br>115 | GGT<br>Gly        | ACA<br>Thr        | AGG<br>Arg        | ACT<br>Thr        | CCT<br>Pro<br>120 | CCC<br>Pro        | ACT<br>Thr        | TTC<br>Phe        | GGC<br>Gly        | GGA<br>Gly<br>125 | GGG<br>Gly        | ACC<br>Thr        | AAG<br>Lys        | 384 |
| GTG<br>Val<br>130 | GAA<br>Glu       | ATC<br>Ile        | AAA<br>Lys        | CGT<br>Arg        | ACG<br>Thr        | GTG<br>Val<br>135 | GCT<br>Ala        | GCA<br>Ala        | CCA<br>Pro        | TCT<br>Ser        | GTC<br>Val<br>140 | TTC<br>Phe        | ATC<br>Ile        | TTC<br>Phe        | CCG<br>Pro        | 432 |
| CCA<br>Pro<br>145 | TCT<br>Ser       | GAT<br>Asp        | GAG<br>Glu        | CAG<br>Gln        | TTG<br>Leu<br>150 | AAA<br>Lys        | TCT<br>Ser        | GGA<br>Gly        | ACT<br>Thr        | GCC<br>Ala<br>155 | TCT<br>Ser        | GTT<br>Val        | GTG<br>Val        | TGC<br>Cys        | CTG<br>Leu<br>160 | 480 |
| CTG<br>Leu        | AAT<br>Asn       | AAC<br>Asn        | TTC<br>Phe        | TAT<br>Tyr<br>165 | CCC<br>Pro        | AGA<br>Arg        | GAG<br>Glu        | GCC<br>Ala        | AAA<br>Lys<br>170 | GTA<br>Val        | CAG<br>Gln        | TGG<br>Trp        | AAG<br>Lys        | GTG<br>Val<br>175 | GAT<br>Asp        | 528 |
| AAC<br>Asn        | GCC<br>Ala       | CTC<br>Leu        | CAA<br>Gln<br>180 | TCG<br>Ser        | GGT<br>Gly        | AAC<br>Asn        | TCC<br>Ser        | CAG<br>Gln<br>185 | GAG<br>Glu        | AGT<br>Ser        | GTC<br>Val        | ACA<br>Thr        | GAG<br>Glu<br>190 | CAG<br>Gln        | GAC<br>Asp        | 576 |
| AGC<br>Ser        | AAG<br>Lys       | GAC<br>Asp<br>195 | AGC<br>Ser        | ACC<br>Thr        | TAC<br>Tyr        | AGC<br>Ser        | CTC<br>Leu<br>200 | AGC<br>Ser        | AGC<br>Ser        | ACC<br>Thr        | CTG<br>Leu        | ACG<br>Thr<br>205 | CTG<br>Leu        | AGC<br>Ser        | AAA<br>Lys        | 624 |
| GCA<br>Ala<br>210 | GAC<br>Asp       | TAC<br>Tyr        | GAG<br>Glu        | AAA<br>Lys        | CAC<br>His        | AAA<br>Lys<br>215 | GTC<br>Val        | TAC<br>Tyr        | GCC<br>Ala        | TGC<br>Cys        | GAA<br>Glu<br>220 | GTC<br>Val        | ACC<br>Thr        | CAT<br>His        | CAG<br>Gln        | 672 |
| GGC               | CTG              | AGC               | TCG               | CCC               | GTC               | ACA               | AAG               | AGC               | TTC               | AAC               | AGG               | GGA               | GAG               | TGT               | TGA               | 720 |

009280-AT68660

(2) INFORMATION FOR SEQ ID NO:6:

(A) LENGTH: 240 amino acids

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln  
210 215 220



Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys \*  
 225 230 235 240

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1437 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1437

## (ix) FEATURE:

- (A) NAME/KEY: mat\_peptide
- (B) LOCATION: 1..1437

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ATG | GGT | TGG | AGC | CTC | ATC | TTG | CTC | TTC | CTT | GTC | GCT | GTT | GCT | ACG | CGT | 48  |
| Met | Gly | Trp | Ser | Leu | Ile | Leu | Leu | Phe | Leu | Val | Ala | Val | Ala | Thr | Arg |     |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
| GTC | CAG | TGT | GAG | GTG | CAA | CTG | GTG | GAG | TCT | GGG | GGA | GGC | TTG | GTC | CAG | 96  |
| Val | Gln | Cys | Glu | Val | Gln | Leu | Val | Glu | Ser | Gly | Gly | Gly | Leu | Val | Gln |     |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
| CCT | GGC | GGG | TCC | CTG | AGA | GTC | TCC | TGT | GCA | GTC | TCT | GGA | TTC | ACC | TTC | 144 |
| Pro | Gly | Gly | Ser | Leu | Arg | Val | Ser | Cys | Ala | Val | Ser | Gly | Phe | Thr | Phe |     |
|     |     |     | 35  |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
| AGT | GAC | CAC | TAC | ATG | TAT | TGG | TTC | CGC | CAG | GCT | CCA | GGG | AAG | GGG | CCG | 192 |
| Ser | Asp | His | Tyr | Met | Tyr | Trp | Phe | Arg | Gln | Ala | Pro | Gly | Lys | Gly | Pro |     |
|     | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |     |
| GAA | TGG | GTA | GGT | TTC | ATT | AGA | AAC | AAA | CCG | AAC | GGT | GGG | ACA | ACA | GAA | 240 |
| Glu | Trp | Val | Gly | Phe | Ile | Arg | Asn | Lys | Pro | Asn | Gly | Gly | Thr | Thr | Glu |     |
| 65  |     |     |     | 70  |     |     |     | 75  |     |     |     |     |     |     | 80  |     |
| TAC | GCC | GCG | TCT | GTG | AAA | GAC | AGA | TTC | ACC | ATC | TCC | AGA | GAT | GAT | TCC | 288 |
| Tyr | Ala | Ala | Ser | Val | Lys | Asp | Arg | Phe | Thr | Ile | Ser | Arg | Asp | Asp | Ser |     |
|     |     |     |     | 85  |     |     |     | 90  |     |     |     |     | 95  |     |     |     |
| AAA | AGC | ATC | GCC | TAT | CTG | CAA | ATG | AGC | AGC | CTG | AAA | ATC | GAG | GAC | ACG | 336 |
| Lys | Ser | Ile | Ala | Tyr | Leu | Gln | Met | Ser | Ser | Leu | Lys | Ile | Glu | Asp | Thr |     |
|     |     |     | 100 |     |     |     | 105 |     |     |     |     |     | 110 |     |     |     |
| GCC | GTC | TAT | TAC | TGT | ACT | ACA | TCC | TAC | ATT | TCA | CAT | TGT | CGG | GGT | GGT | 384 |
| Ala | Val | Tyr | Tyr | Cys | Thr | Thr | Ser | Tyr | Ile | Ser | His | Cys | Arg | Gly | Gly |     |

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| 115 |     |     | 120 |     |     | 125 |     |     |     |     |     |     |     |     |     |  |  |  |  |      |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|--|------|
| GTC | TGC | TAT | GGA | GGT | TAC | TTC | GAA | TTC | TGG | GGC | CAG | GGC | GCC | CTG | GTC |  |  |  |  | 432  |
| Val | Cys | Tyr | Gly | Gly | Tyr | Phe | Glu | Phe | Trp | Gly | Gln | Gly | Ala | Leu | Val |  |  |  |  |      |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |  |  |  |      |
| ACC | GTC | TCC | TCA | GCT | AGC | ACC | AAG | GGC | CCA | TCG | GTC | TTC | CCC | CTG | GCA |  |  |  |  | 480  |
| Thr | Val | Ser | Ser | Ala | Ser | Thr | Lys | Gly | Pro | Ser | Val | Phe | Pro | Leu | Ala |  |  |  |  |      |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |  |  |  |      |
| CCC | TCC | TCC | AAG | AGC | ACC | TCT | GGG | GGC | ACA | GCG | GCC | CTG | GGC | TGC | CTG |  |  |  |  | 528  |
| Pro | Ser | Ser | Lys | Ser | Thr | Ser | Gly | Gly | Thr | Ala | Ala | Leu | Gly | Cys | Leu |  |  |  |  |      |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |  |  |  |      |
| GTC | AAG | GAC | TAC | TTC | CCC | GAA | CCG | GTG | ACG | GTG | TCG | TGG | AAC | TCA | GGC |  |  |  |  | 576  |
| Val | Lys | Asp | Tyr | Phe | Pro | Glu | Pro | Val | Thr | Val | Ser | Trp | Asn | Ser | Gly |  |  |  |  |      |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |  |  |  |      |
| GCC | CTG | ACC | AGC | GGC | GTG | CAC | ACC | TTC | CCG | GCT | GTC | CTA | CAG | TCC | TCA |  |  |  |  | 624  |
| Ala | Leu | Thr | Ser | Gly | Val | His | Thr | Phe | Pro | Ala | Val | Leu | Gln | Ser | Ser |  |  |  |  |      |
|     |     |     | 195 |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |  |  |  |      |
| GGA | CTC | TAC | TCC | CTC | AGC | AGC | GTG | GTG | ACC | GTG | CCC | TCC | AGC | AGC | TTG |  |  |  |  | 672  |
| Gly | Leu | Tyr | Ser | Leu | Ser | Ser | Val | Val | Thr | Val | Pro | Ser | Ser | Ser | Leu |  |  |  |  |      |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |  |  |  |  |      |
| GGC | ACC | CAG | ACC | TAC | ATC | TGC | AAC | GTG | AAT | CAC | AAG | CCC | AGC | AAC | ACC |  |  |  |  | 720  |
| Gly | Thr | Gln | Thr | Tyr | Ile | Cys | Asn | Val | Asn | His | Lys | Pro | Ser | Asn | Thr |  |  |  |  |      |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |  |  |  |      |
| AAG | GTG | GAC | AAG | AAA | GCA | GAG | CCC | AAA | TCT | TGT | GAC | AAA | ACT | CAC | ACA |  |  |  |  | 768  |
| Lys | Val | Asp | Lys | Lys | Ala | Glu | Pro | Lys | Ser | Cys | Asp | Lys | Thr | His | Thr |  |  |  |  |      |
|     |     |     |     | 245 |     |     |     | 250 |     |     |     |     | 255 |     |     |  |  |  |  |      |
| TGC | CCA | CCG | TGC | CCA | GCA | CCT | GAA | CTC | CTG | GGG | GGA | CCG | TCA | GTC | TTC |  |  |  |  | 816  |
| Cys | Pro | Pro | Cys | Pro | Ala | Pro | Glu | Leu | Leu | Gly | Gly | Pro | Ser | Val | Phe |  |  |  |  |      |
|     |     |     | 260 |     |     |     | 265 |     |     |     |     |     | 270 |     |     |  |  |  |  |      |
| CTC | TTC | CCC | CCA | AAA | CCC | AAG | GAC | ACC | CTC | ATG | ATC | TCC | CGG | ACC | CCT |  |  |  |  | 864  |
| Leu | Phe | Pro | Pro | Lys | Pro | Lys | Asp | Thr | Leu | Met | Ile | Ser | Arg | Thr | Pro |  |  |  |  |      |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |  |  |  |  |      |
| GAG | GTC | ACA | TGC | GTG | GTG | GTG | GAC | GTG | AGC | CAC | GAA | GAC | CCT | GAG | GTC |  |  |  |  | 912  |
| Glu | Val | Thr | Cys | Val | Val | Val | Asp | Val | Ser | His | Glu | Asp | Pro | Glu | Val |  |  |  |  |      |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |  |  |  |  |      |
| AAG | TTC | AAC | TGG | TAC | GTG | GAC | GGC | GTG | GAG | GTG | CAT | AAT | GCC | AAG | ACA |  |  |  |  | 960  |
| Lys | Phe | Asn | Trp | Tyr | Val | Asp | Gly | Val | Glu | Val | His | Asn | Ala | Lys | Thr |  |  |  |  |      |
| 305 |     |     |     |     | 310 |     |     |     | 315 |     |     |     |     |     | 320 |  |  |  |  |      |
| AAG | CCG | CGG | GAG | GAG | CAG | TAC | AAC | AGC | ACG | TAC | CGT | GTG | GTC | AGC | GTC |  |  |  |  | 1008 |
| Lys | Pro | Arg | Glu | Glu | Gln | Tyr | Asn | Ser | Thr | Tyr | Arg | Val | Val | Ser | Val |  |  |  |  |      |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |  |  |  |  |      |
| CTC | ACC | GTC | CTG | CAC | CAG | GAC | TGG | CTG | AAT | GGC | AAG | GAG | TAC | AAG | TGC |  |  |  |  | 1056 |
| Leu | Thr | Val | Leu | His | Gln | Asp | Trp | Leu | Asn | Gly | Lys | Glu | Tyr | Lys | Cys |  |  |  |  |      |

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| 340 |     |     |     |     |     | 345 |     |     |     |     |     | 350 |     |     |     |      |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|--|--|
| AAG | GTC | TCC | AAC | AAA | GCC | CTC | CCA | GCC | CCC | ATC | GAG | AAA | ACC | ATC | TCC | 1104 |  |  |
| Lys | Val | Ser | Asn | Lys | Ala | Leu | Pro | Ala | Pro | Ile | Glu | Lys | Thr | Ile | Ser |      |  |  |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |      |  |  |
| AAA | GCC | AAA | GGG | CAG | CCC | CGA | GAA | CCA | CAG | GTG | TAC | ACC | CTG | CCC | CCA | 1152 |  |  |
| Lys | Ala | Lys | Gly | Gln | Pro | Arg | Glu | Pro | Gln | Val | Tyr | Thr | Leu | Pro | Pro |      |  |  |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |      |  |  |
| TCC | CGG | GAT | GAG | CTG | ACC | AAG | AAC | CAG | GTC | AGC | CTG | ACC | TGC | CTG | GTC | 1200 |  |  |
| Ser | Arg | Asp | Glu | Leu | Thr | Lys | Asn | Gln | Val | Ser | Leu | Thr | Cys | Leu | Val |      |  |  |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |      |  |  |
| AAA | GGC | TTC | TAT | CCC | AGC | GAC | ATC | GCC | GTG | GAG | TGG | GAG | AGC | AAT | GGG | 1248 |  |  |
| Lys | Gly | Phe | Tyr | Pro | Ser | Asp | Ile | Ala | Val | Glu | Trp | Glu | Ser | Asn | Gly |      |  |  |
|     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |      |  |  |
| CAG | CCG | GAG | AAC | AAC | TAC | AAG | ACC | ACG | CCT | CCC | GTG | CTG | GAC | TCC | GAC | 1296 |  |  |
| Gln | Pro | Glu | Asn | Asn | Tyr | Lys | Thr | Thr | Pro | Pro | Val | Leu | Asp | Ser | Asp |      |  |  |
|     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |      |  |  |
| GGC | TCC | TTC | TTC | CTC | TAC | AGC | AAG | CTC | ACC | GTG | GAC | AAG | AGC | AGG | TGG | 1344 |  |  |
| Gly | Ser | Phe | Phe | Leu | Tyr | Ser | Lys | Leu | Thr | Val | Asp | Lys | Ser | Arg | Trp |      |  |  |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |      |  |  |
| CAG | CAG | GGG | AAC | GTC | TTC | TCA | TGC | TCC | GTG | ATG | CAT | GAG | GCT | CTG | CAC | 1392 |  |  |
| Gln | Gln | Gly | Asn | Val | Phe | Ser | Cys | Ser | Val | Met | His | Glu | Ala | Leu | His |      |  |  |
|     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |      |  |  |
| AAC | CAC | TAC | ACG | CAG | AAG | AGC | CTC | TCC | CTG | TCT | CCG | GGT | AAA | TGA |     | 1437 |  |  |
| Asn | His | Tyr | Thr | Gln | Lys | Ser | Leu | Ser | Leu | Ser | Pro | Gly | Lys | *   |     |      |  |  |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     |     |      |  |  |

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 479 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Met Gly Trp Ser Leu Ile Leu Leu Phe Leu Val Ala Val Ala Thr Arg
 1           5           10           15

Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
 20           25           30

Pro Gly Gly Ser Leu Arg Val Ser Cys Ala Val Ser Gly Phe Thr Phe
 35           40           45

Ser Asp His Tyr Met Tyr Trp Phe Arg Gln Ala Pro Gly Lys Gly Pro

```

| 50         |            |            |            |            | 55         |            |            |            |            | 60         |            |            |            |            |            |
|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Glu<br>65  | Trp        | Val        | Gly        | Phe        | Ile<br>70  | Arg        | Asn        | Lys        | Pro        | Asn<br>75  | Gly        | Gly        | Thr        | Thr        | Glu<br>80  |
| Tyr        | Ala        | Ala        | Ser        | Val<br>85  | Lys        | Asp        | Arg        | Phe        | Thr<br>90  | Ile        | Ser        | Arg        | Asp        | Asp<br>95  | Ser        |
| Lys        | Ser        | Ile        | Ala<br>100 | Tyr        | Leu        | Gln        | Met        | Ser<br>105 | Ser        | Leu        | Lys        | Ile        | Glu<br>110 | Asp        | Thr        |
| Ala        | Val        | Tyr<br>115 | Tyr        | Cys        | Thr        | Thr        | Ser<br>120 | Tyr        | Ile        | Ser        | His        | Cys<br>125 | Arg        | Gly        | Gly        |
| Val        | Cys<br>130 | Tyr        | Gly        | Gly        | Tyr        | Phe<br>135 | Glu        | Phe        | Trp        | Gly        | Gln<br>140 | Gly        | Ala        | Leu        | Val        |
| Thr<br>145 | Val        | Ser        | Ser        | Ala        | Ser<br>150 | Thr        | Lys        | Gly        | Pro        | Ser<br>155 | Val        | Phe        | Pro        | Leu        | Ala<br>160 |
| Pro        | Ser        | Ser        | Lys        | Ser<br>165 | Thr        | Ser        | Gly        | Gly        | Thr<br>170 | Ala        | Ala        | Leu        | Gly        | Cys<br>175 | Leu        |
| Val        | Lys        | Asp        | Tyr<br>180 | Phe        | Pro        | Glu        | Pro<br>185 | Val        | Thr        | Val        | Ser        | Trp        | Asn<br>190 | Ser        | Gly        |
| Ala        | Leu        | Thr<br>195 | Ser        | Gly        | Val        | His<br>200 | Thr        | Phe        | Pro        | Ala        | Val<br>205 | Leu        | Gln        | Ser        | Ser        |
| Gly<br>210 | Leu        | Tyr        | Ser        | Leu        | Ser        | Ser<br>215 | Val        | Val        | Thr        | Val        | Pro<br>220 | Ser        | Ser        | Ser        | Leu        |
| Gly<br>225 | Thr        | Gln        | Thr        | Tyr        | Ile<br>230 | Cys        | Asn        | Val        | Asn        | His<br>235 | Lys        | Pro        | Ser        | Asn        | Thr<br>240 |
| Lys        | Val        | Asp        | Lys        | Lys<br>245 | Ala        | Glu        | Pro        | Lys        | Ser<br>250 | Cys        | Asp        | Lys        | Thr        | His<br>255 | Thr        |
| Cys        | Pro        | Pro        | Cys<br>260 | Pro        | Ala        | Pro        | Glu        | Leu<br>265 | Leu        | Gly        | Gly        | Pro        | Ser<br>270 | Val        | Phe        |
| Leu        | Phe<br>275 | Pro        | Pro        | Lys        | Pro        | Lys        | Asp<br>280 | Thr        | Leu        | Met        | Ile        | Ser<br>285 | Arg        | Thr        | Pro        |
| Glu<br>290 | Val        | Thr        | Cys        | Val        | Val        | Val<br>295 | Asp        | Val        | Ser        | His        | Glu<br>300 | Asp        | Pro        | Glu        | Val        |
| Lys<br>305 | Phe        | Asn        | Trp        | Tyr        | Val<br>310 | Asp        | Gly        | Val        | Glu        | Val<br>315 | His        | Asn        | Ala        | Lys        | Thr<br>320 |
| Lys        | Pro        | Arg        | Glu        | Glu<br>325 | Gln        | Tyr        | Asn        | Ser        | Thr<br>330 | Tyr        | Arg        | Val        | Val        | Ser<br>335 | Val        |
| Leu        | Thr        | Val        | Leu<br>340 | His        | Gln        | Asp        | Trp        | Leu<br>345 | Asn        | Gly        | Lys        | Glu        | Tyr<br>350 | Lys        | Cys        |

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Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser  
 355 360 365  
 Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro  
 370 375 380  
 Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val  
 385 390 395 400  
 Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
 405 410 415  
 Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
 420 425 430  
 Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp  
 435 440 445  
 Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
 450 455 460  
 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys \*  
 465 470 475

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 711 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..711

## (ix) FEATURE:

- (A) NAME/KEY: mat\_peptide
- (B) LOCATION: 1..711

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ATG | AGG | GTC | CCC | GCT | CAG | CTC | CTG | GGG | CTC | CTG | CTG | CTC | TGG | CTC | CCA | 48  |
| Met | Arg | Val | Pro | Ala | Gln | Leu | Leu | Gly | Leu | Leu | Leu | Leu | Trp | Leu | Pro |     |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     | 15  |     |     |     |
| GGT | GCA | CGA | TGT | GAG | TCT | GTC | CTG | ACA | CAG | CCG | CCC | TCA | GTG | TCT | GGG | 96  |
| Gly | Ala | Arg | Cys | Glu | Ser | Val | Leu | Thr | Gln | Pro | Pro | Ser | Val | Ser | Gly |     |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
| GCC | CCA | GGG | CAG | AAG | GTC | ACC | ATC | TCG | TGC | ACT | GGG | AGC | ACC | TCC | AAC | 144 |
| Ala | Pro | Gly | Gln | Lys | Val | Thr | Ile | Ser | Cys | Thr | Gly | Ser | Thr | Ser | Asn |     |

| 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |     |     |     |  |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|
| ATT | GGA | GGT | TAT | GAT | CTA | CAT | TGG | TAC | CAG | CAG | CTC | CCA | GGA | ACG | GCC | 192 |     |  |  |  |
| Ile | Gly | Gly | Tyr | Asp | Leu | His | Trp | Tyr | Gln | Gln | Leu | Pro | Gly | Thr | Ala |     |     |  |  |  |
| 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |     |     |     |  |  |  |
| CCC | AAA | CTC | CTC | ATC | TAT | GAC | ATT | AAC | AAG | CGA | CCC | TCA | GGA | ATT | TCT | 240 |     |  |  |  |
| Pro | Lys | Leu | Leu | Ile | Tyr | Asp | Ile | Asn | Lys | Arg | Pro | Ser | Gly | Ile | Ser |     |     |  |  |  |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |     |     |  |  |  |
| GAC | CGA | TTC | TCT | GGC | TCC | AAG | TCT | GGT | ACC | GCG | GCC | TCC | CTG | GCC | ATC | 288 |     |  |  |  |
| Asp | Arg | Phe | Ser | Gly | Ser | Lys | Ser | Gly | Thr | Ala | Ala | Ser | Leu | Ala | Ile |     |     |  |  |  |
| 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |     |     |     |     |     |     |  |  |  |
| ACT | GGG | CTC | CAG | ACT | GAG | GAT | GAG | GCT | GAT | TAT | TAC | TGC | CAG | TCC | TAT | 336 |     |  |  |  |
| Thr | Gly | Leu | Gln | Thr | Glu | Asp | Glu | Ala | Asp | Tyr | Tyr | Cys | Gln | Ser | Tyr |     |     |  |  |  |
| 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |     |     |     |     |  |  |  |
| GAC | AGC | AGC | CTG | AAT | GCT | CAG | GTA | TTC | GGA | GGA | GGG | ACC | CGG | CTG | ACC | 384 |     |  |  |  |
| Asp | Ser | Ser | Leu | Asn | Ala | Gln | Val | Phe | Gly | Gly | Gly | Thr | Arg | Leu | Thr |     |     |  |  |  |
| 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |     |     |     |  |  |  |
| GTC | CTA | GGT | CAG | CCC | AAG | GCT | GCC | CCC | TCG | GTC | ACT | CTG | TTC | CCG | CCC | 432 |     |  |  |  |
| Val | Leu | Gly | Gln | Pro | Lys | Ala | Ala | Pro | Ser | Val | Thr | Leu | Phe | Pro | Pro |     |     |  |  |  |
| 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |     |     |     |  |  |  |
| TCC | TCT | GAG | GAG | CTT | CAA | GCC | AAC | AAG | GCC | ACA | CTG | GTG | TGT | CTC | ATA | 480 |     |  |  |  |
| Ser | Ser | Glu | Glu | Leu | Gln | Ala | Asn | Lys | Ala | Thr | Leu | Val | Cys | Leu | Ile |     |     |  |  |  |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |     |  |  |  |
| AGT | GAC | TTC | TAC | CCG | GGA | GCC | GTG | ACA | GTG | GCC | TGG | AAG | GCA | GAT | AGC | 528 |     |  |  |  |
| Ser | Asp | Phe | Tyr | Pro | Gly | Ala | Val | Thr | Val | Ala | Trp | Lys | Ala | Asp | Ser |     |     |  |  |  |
| 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     |     |     |     |  |  |  |
| AGC | CCC | GTC | AAG | GCG | GGA | GTG | GAG | ACC | ACC | ACA | CCC | TCC | AAA | CAA | AGC | 576 |     |  |  |  |
| Ser | Pro | Val | Lys | Ala | Gly | Val | Glu | Thr | Thr | Thr | Pro | Ser | Lys | Gln | Ser |     |     |  |  |  |
| 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |     |     |     |     |  |  |  |
| AAC | AAC | AAG | TAC | GCG | GCC | AGC | AGC | TAC | CTG | AGC | CTG | ACG | CCT | GAG | CAG | 624 |     |  |  |  |
| Asn | Asn | Lys | Tyr | Ala | Ala | Ser | Ser | Tyr | Leu | Ser | Leu | Thr | Pro | Glu | Gln |     |     |  |  |  |
| 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |     |     |     |  |  |  |
| TGG | AAG | TCC | CAC | AGA | AGC | TAC | AGC | TGC | CAG | GTC | ACG | CAT | GAA | GGG | AGC | 672 |     |  |  |  |
| Trp | Lys | Ser | His | Arg | Ser | Tyr | Ser | Cys | Gln | Val | Thr | His | Glu | Gly | Ser |     |     |  |  |  |
| 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |     |     |     |  |  |  |
| ACC | GTG | GAG | AAG | ACA | GTG | GCC | CCT | ACA | GAA | TGT | TCA | TGA |     |     |     |     | 711 |  |  |  |
| Thr | Val | Glu | Lys | Thr | Val | Ala | Pro | Thr | Glu | Cys | Ser | *   |     |     |     |     |     |  |  |  |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     |     |     |     |  |  |  |

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 237 amino acids  
 (B) TYPE: amino acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

|            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Met<br>1   | Arg        | Val        | Pro        | Ala<br>5   | Gln        | Leu        | Leu        | Gly        | Leu<br>10  | Leu        | Leu        | Leu        | Trp        | Leu<br>15  | Pro        |
| Gly        | Ala        | Arg        | Cys<br>20  | Glu        | Ser        | Val        | Leu        | Thr<br>25  | Gln        | Pro        | Pro        | Ser        | Val<br>30  | Ser        | Gly        |
| Ala        | Pro        | Gly<br>35  | Gln        | Lys        | Val        | Thr        | Ile<br>40  | Ser        | Cys        | Thr        | Gly        | Ser<br>45  | Thr        | Ser        | Asn        |
| Ile        | Gly<br>50  | Gly        | Tyr        | Asp        | Leu        | His<br>55  | Trp        | Tyr        | Gln        | Gln        | Leu<br>60  | Pro        | Gly        | Thr        | Ala        |
| Pro<br>65  | Lys        | Leu        | Leu        | Ile        | Tyr<br>70  | Asp        | Ile        | Asn        | Lys        | Arg<br>75  | Pro        | Ser        | Gly        | Ile        | Ser<br>80  |
| Asp        | Arg        | Phe        | Ser        | Gly<br>85  | Ser        | Lys        | Ser        | Gly        | Thr<br>90  | Ala        | Ala        | Ser        | Leu        | Ala<br>95  | Ile        |
| Thr        | Gly        | Leu        | Gln<br>100 | Thr        | Glu        | Asp        | Glu        | Ala<br>105 | Asp        | Tyr        | Tyr        | Cys        | Gln<br>110 | Ser        | Tyr        |
| Asp        | Ser        | Ser<br>115 | Leu        | Asn        | Ala        | Gln        | Val<br>120 | Phe        | Gly        | Gly        | Gly        | Thr<br>125 | Arg        | Leu        | Thr        |
| Val        | Leu<br>130 | Gly        | Gln        | Pro        | Lys        | Ala<br>135 | Ala        | Pro        | Ser        | Val        | Thr<br>140 | Leu        | Phe        | Pro        | Pro        |
| Ser<br>145 | Ser        | Glu        | Glu        | Leu        | Gln<br>150 | Ala        | Asn        | Lys        | Ala        | Thr<br>155 | Leu        | Val        | Cys        | Leu        | Ile<br>160 |
| Ser        | Asp        | Phe        | Tyr        | Pro<br>165 | Gly        | Ala        | Val        | Thr        | Val<br>170 | Ala        | Trp        | Lys        | Ala        | Asp<br>175 | Ser        |
| Ser        | Pro        | Val        | Lys<br>180 | Ala        | Gly        | Val        | Glu        | Thr<br>185 | Thr        | Thr        | Pro        | Ser        | Lys<br>190 | Gln        | Ser        |
| Asn        | Asn        | Lys<br>195 | Tyr        | Ala        | Ala        | Ser        | Ser<br>200 | Tyr        | Leu        | Ser        | Leu        | Thr<br>205 | Pro        | Glu        | Gln        |
| Trp        | Lys<br>210 | Ser        | His        | Arg        | Ser        | Tyr<br>215 | Ser        | Cys        | Gln        | Val        | Thr<br>220 | His        | Glu        | Gly        | Ser        |
| Thr<br>225 | Val        | Glu        | Lys        | Thr        | Val<br>230 | Ala        | Pro        | Thr        | Glu        | Cys<br>235 | Ser        | *          |            |            |            |

(B) TYPE: nucleic acid

(ii) MOLECULE TYPE: peptide

(A) NAME/KEY: CDS  
(B) LOCATION: 1..1431

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(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..1431
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|                   |                   |                   |                   |                  |                   |                   |                   |                   |                  |                   |                   |                   |                   |                  |                   |     |
|-------------------|-------------------|-------------------|-------------------|------------------|-------------------|-------------------|-------------------|-------------------|------------------|-------------------|-------------------|-------------------|-------------------|------------------|-------------------|-----|
| ATG<br>Met<br>1   | AAA<br>Lys        | CAC<br>His        | CTG<br>Leu        | TGG<br>Trp<br>5  | TTC<br>Phe        | TTC<br>Phe        | CTC<br>Leu        | CTC<br>Leu        | CTG<br>Leu<br>10 | GTG<br>Val        | GCA<br>Ala        | GCT<br>Ala        | CCC<br>Pro        | AGA<br>Arg<br>15 | TGG<br>Trp        | 48  |
| GTC<br>Val        | CTG<br>Leu        | TCC<br>Ser        | CAG<br>Gln<br>20  | GTG<br>Val       | CAG<br>Gln        | CTG<br>Leu        | CAG<br>Gln        | GAG<br>Glu<br>25  | TCG<br>Ser       | GGC<br>Gly        | CCA<br>Pro        | GGA<br>Gly        | CTG<br>Leu<br>30  | GTG<br>Val       | AAG<br>Lys        | 96  |
| CCT<br>Pro        | TCG<br>Ser        | GAG<br>Glu<br>35  | ACC<br>Thr        | CTG<br>Leu       | TCC<br>Ser        | CTC<br>Leu        | ACC<br>Thr<br>40  | TGC<br>Cys        | GCT<br>Ala       | GTG<br>Val        | TCT<br>Ser        | GGT<br>Gly<br>45  | GGC<br>Gly        | TCC<br>Ser       | ATC<br>Ile        | 144 |
| AGC<br>Ser<br>50  | GGT<br>Gly        | GGT<br>Gly        | TAT<br>Tyr        | GGC<br>Gly       | TGG<br>Trp        | GGC<br>Gly<br>55  | TGG<br>Trp        | ATC<br>Ile        | CGC<br>Arg       | CAG<br>Gln        | CCC<br>Pro<br>60  | CCA<br>Pro        | GGG<br>Gly        | AAG<br>Lys       | GGG<br>Gly        | 192 |
| CTG<br>Leu<br>65  | GAG<br>Glu        | TGG<br>Trp        | ATT<br>Ile        | GGG<br>Gly       | AGT<br>Ser<br>70  | TTC<br>Phe        | TAT<br>Tyr        | AGT<br>Ser        | AGT<br>Ser       | AGT<br>Ser<br>75  | GGG<br>Gly        | AAC<br>Asn        | ACC<br>Thr        | TAC<br>Tyr       | TAC<br>Tyr<br>80  | 240 |
| AAC<br>Asn        | CCC<br>Pro        | TCC<br>Ser        | CTC<br>Leu        | AAG<br>Lys<br>85 | AGT<br>Ser        | CAA<br>Gln        | GTC<br>Val        | ACC<br>Thr        | ATT<br>Ile<br>90 | TCA<br>Ser        | ACA<br>Thr        | GAC<br>Asp        | ACG<br>Thr        | TCC<br>Ser<br>95 | AAG<br>Lys        | 288 |
| AAC<br>Asn        | CAG<br>Gln        | TTC<br>Phe        | TCC<br>Ser<br>100 | CTG<br>Leu       | AAG<br>Lys        | CTG<br>Leu        | AAC<br>Asn        | TCT<br>Ser<br>105 | ATG<br>Met       | ACC<br>Thr        | GCC<br>Ala        | GCG<br>Ala        | GAC<br>Asp<br>110 | ACG<br>Thr       | GCC<br>Ala        | 336 |
| GTG<br>Val        | TAT<br>Tyr        | TAC<br>Tyr<br>115 | TGT<br>Cys        | GTG<br>Val       | AGA<br>Arg        | GAT<br>Asp        | CGT<br>Arg<br>120 | CTT<br>Leu        | TTT<br>Phe       | TCA<br>Ser        | GTT<br>Val        | GTT<br>Val<br>125 | GGA<br>Gly        | ATG<br>Met       | GTT<br>Val        | 384 |
| TAC<br>Tyr        | AAC<br>Asn<br>130 | AAC<br>Asn        | TGG<br>Trp        | TTC<br>Phe       | GAT<br>Asp        | GTC<br>Val<br>135 | TGG<br>Trp        | GGC<br>Gly        | CCG<br>Pro       | GGA<br>Gly        | GTC<br>Val<br>140 | CTG<br>Leu        | GTC<br>Val        | ACC<br>Thr       | GTC<br>Val        | 432 |
| TCC<br>Ser<br>145 | TCA<br>Ser        | GCT<br>Ala        | AGC<br>Ser        | ACC<br>Thr       | AAG<br>Lys<br>150 | GGC<br>Gly        | CCA<br>Pro        | TCG<br>Ser        | GTC<br>Val       | TTC<br>Phe<br>155 | CCC<br>Pro        | CTG<br>Leu        | GCA<br>Ala        | CCC<br>Pro       | TCC<br>Ser<br>160 | 480 |



|                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |      |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| TCC<br>Ser        | AAG<br>Lys        | AGC<br>Ser        | ACC<br>Thr        | TCT<br>Ser<br>165 | GGG<br>Gly        | GGC<br>Gly        | ACA<br>Thr        | GCG<br>Ala        | GCC<br>Ala<br>170 | CTG<br>Leu        | GGC<br>Gly        | TGC<br>Cys        | CTG<br>Leu        | GTC<br>Val<br>175 | AAG<br>Lys        | 528  |
| GAC<br>Asp        | TAC<br>Tyr        | TTC<br>Phe        | CCC<br>Pro<br>180 | GAA<br>Glu        | CCG<br>Pro        | GTG<br>Val        | ACG<br>Thr        | GTG<br>Val<br>185 | TCG<br>Ser        | TGG<br>Trp        | AAC<br>Asn        | TCA<br>Ser        | GGC<br>Gly<br>190 | GCC<br>Ala        | CTG<br>Leu        | 576  |
| ACC<br>Thr        | AGC<br>Ser        | GGC<br>Gly<br>195 | GTG<br>Val        | CAC<br>His        | ACC<br>Thr        | TTC<br>Phe        | CCG<br>Pro<br>200 | GCT<br>Ala        | GTC<br>Val        | CTA<br>Leu        | CAG<br>Gln        | TCC<br>Ser<br>205 | TCA<br>Ser        | GGA<br>Gly        | CTC<br>Leu        | 624  |
| TAC<br>Tyr        | TCC<br>Ser<br>210 | CTC<br>Leu        | AGC<br>Ser        | AGC<br>Ser        | GTG<br>Val        | GTG<br>Val<br>215 | ACC<br>Thr        | GTG<br>Val        | CCC<br>Pro        | TCC<br>Ser        | AGC<br>Ser<br>220 | AGC<br>Ser        | TTG<br>Leu        | GGC<br>Gly        | ACC<br>Thr        | 672  |
| CAG<br>Gln<br>225 | ACC<br>Thr        | TAC<br>Tyr        | ATC<br>Ile        | TGC<br>Cys        | AAC<br>Asn<br>230 | GTG<br>Val        | AAT<br>Asn        | CAC<br>His        | AAG<br>Lys        | CCC<br>Pro<br>235 | AGC<br>Ser        | AAC<br>Asn        | ACC<br>Thr        | AAG<br>Lys<br>240 | GTG<br>Val        | 720  |
| GAC<br>Asp        | AAG<br>Lys        | AAA<br>Lys        | GCA<br>Ala        | GAG<br>Glu<br>245 | CCC<br>Pro        | AAA<br>Lys        | TCT<br>Ser        | TGT<br>Cys        | GAC<br>Asp<br>250 | AAA<br>Lys        | ACT<br>Thr        | CAC<br>His        | ACA<br>Thr        | TGC<br>Cys<br>255 | CCA<br>Pro        | 768  |
| CCG<br>Pro        | TGC<br>Cys        | CCA<br>Pro        | GCA<br>Ala<br>260 | CCT<br>Pro        | GAA<br>Glu        | CTC<br>Leu        | CTG<br>Leu        | GGG<br>Gly<br>265 | GGA<br>Gly        | CCG<br>Pro        | TCA<br>Ser        | GTC<br>Val        | TTC<br>Phe<br>270 | CTC<br>Leu        | TTC<br>Phe        | 816  |
| CCC<br>Pro        | CCA<br>Pro        | AAA<br>Lys<br>275 | CCC<br>Pro        | AAG<br>Lys        | GAC<br>Asp        | ACC<br>Thr        | CTC<br>Leu<br>280 | ATG<br>Met        | ATC<br>Ile        | TCC<br>Ser        | CGG<br>Arg        | ACC<br>Thr<br>285 | CCT<br>Pro        | GAG<br>Glu        | GTC<br>Val        | 864  |
| ACA<br>Thr        | TGC<br>Cys<br>290 | GTG<br>Val        | GTG<br>Val        | GTG<br>Val        | GAC<br>Asp        | GTG<br>Val<br>295 | AGC<br>Ser        | CAC<br>His        | GAA<br>Glu        | GAC<br>Asp        | CCT<br>Pro<br>300 | GAG<br>Glu        | GTC<br>Val        | AAG<br>Lys        | TTC<br>Phe        | 912  |
| AAC<br>Asn<br>305 | TGG<br>Trp        | TAC<br>Tyr        | GTG<br>Val        | GAC<br>Asp        | GGC<br>Gly<br>310 | GTG<br>Val        | GAG<br>Glu        | GTG<br>Val        | CAT<br>His        | AAT<br>Asn<br>315 | GCC<br>Ala        | AAG<br>Lys        | ACA<br>Thr        | AAG<br>Lys        | CCG<br>Pro<br>320 | 960  |
| CGG<br>Arg        | GAG<br>Glu        | GAG<br>Glu        | CAG<br>Gln        | TAC<br>Tyr<br>325 | AAC<br>Asn        | AGC<br>Ser        | ACG<br>Thr        | TAC<br>Tyr        | CGT<br>Arg<br>330 | GTG<br>Val        | GTC<br>Val        | AGC<br>Ser        | GTC<br>Val        | CTC<br>Leu<br>335 | ACC<br>Thr        | 1008 |
| GTC<br>Val        | CTG<br>Leu        | CAC<br>His        | CAG<br>Gln<br>340 | GAC<br>Asp        | TGG<br>Trp        | CTG<br>Leu        | AAT<br>Asn        | GGC<br>Gly<br>345 | AAG<br>Lys        | GAG<br>Glu        | TAC<br>Tyr        | AAG<br>Lys        | TGC<br>Cys<br>350 | AAG<br>Lys        | GTC<br>Val        | 1056 |
| TCC<br>Ser        | AAC<br>Asn        | AAA<br>Lys<br>355 | GCC<br>Ala        | CTC<br>Leu        | CCA<br>Pro        | GCC<br>Ala        | CCC<br>Pro<br>360 | ATC<br>Ile        | GAG<br>Glu        | AAA<br>Lys        | ACC<br>Thr        | ATC<br>Ile<br>365 | TCC<br>Ser        | AAA<br>Lys        | GCC<br>Ala        | 1104 |
| AAA<br>Lys        | GGG<br>Gly<br>370 | CAG<br>Gln        | CCC<br>Pro        | CGA<br>Arg        | GAA<br>Glu        | CCA<br>Pro<br>375 | CAG<br>Gln        | GTG<br>Val        | TAC<br>Tyr        | ACC<br>Thr        | CTG<br>Leu<br>380 | CCC<br>Pro        | CCA<br>Pro        | TCC<br>Ser        | CGG<br>Arg        | 1152 |

GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC 1200  
 Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly  
 385 390 395 400  
 TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG 1248  
 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro  
 405 410 415  
 GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC 1296  
 Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser  
 420 425 430  
 TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG 1344  
 Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln  
 435 440 445  
 GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC 1392  
 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His  
 450 455 460  
 TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA 1431  
 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys \*  
 465 470 475

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 477 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp  
 1 5 10 15  
 Val Leu Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys  
 20 25 30  
 Pro Ser Glu Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Gly Ser Ile  
 35 40 45  
 Ser Gly Gly Tyr Gly Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly  
 50 55 60  
 Leu Glu Trp Ile Gly Ser Phe Tyr Ser Ser Ser Gly Asn Thr Tyr Tyr  
 65 70 75 80  
 Asn Pro Ser Leu Lys Ser Gln Val Thr Ile Ser Thr Asp Thr Ser Lys  
 85 90 95  
 Asn Gln Phe Ser Leu Lys Leu Asn Ser Met Thr Ala Ala Asp Thr Ala  
 100 105 110

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Tyr | Tyr | Cys | Val | Arg | Asp | Arg | Leu | Phe | Ser | Val | Val | Gly | Met | Val |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Tyr | Asn | Asn | Trp | Phe | Asp | Val | Trp | Gly | Pro | Gly | Val | Leu | Val | Thr | Val |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Ser | Ser | Ala | Ser | Thr | Lys | Gly | Pro | Ser | Val | Phe | Pro | Leu | Ala | Pro | Ser |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
| Ser | Lys | Ser | Thr | Ser | Gly | Gly | Thr | Ala | Ala | Leu | Gly | Cys | Leu | Val | Lys |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Asp | Tyr | Phe | Pro | Glu | Pro | Val | Thr | Val | Ser | Trp | Asn | Ser | Gly | Ala | Leu |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Thr | Ser | Gly | Val | His | Thr | Phe | Pro | Ala | Val | Leu | Gln | Ser | Ser | Gly | Leu |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Tyr | Ser | Leu | Ser | Ser | Val | Val | Thr | Val | Pro | Ser | Ser | Ser | Leu | Gly | Thr |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Gln | Thr | Tyr | Ile | Cys | Asn | Val | Asn | His | Lys | Pro | Ser | Asn | Thr | Lys | Val |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Asp | Lys | Lys | Ala | Glu | Pro | Lys | Ser | Cys | Asp | Lys | Thr | His | Thr | Cys | Pro |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Pro | Cys | Pro | Ala | Pro | Glu | Leu | Leu | Gly | Gly | Pro | Ser | Val | Phe | Leu | Phe |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Pro | Pro | Lys | Pro | Lys | Asp | Thr | Leu | Met | Ile | Ser | Arg | Thr | Pro | Glu | Val |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| Thr | Cys | Val | Val | Val | Asp | Val | Ser | His | Glu | Asp | Pro | Glu | Val | Lys | Phe |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Asn | Trp | Tyr | Val | Asp | Gly | Val | Glu | Val | His | Asn | Ala | Lys | Thr | Lys | Pro |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| Arg | Glu | Glu | Gln | Tyr | Asn | Ser | Thr | Tyr | Arg | Val | Val | Ser | Val | Leu | Thr |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
| Val | Leu | His | Gln | Asp | Trp | Leu | Asn | Gly | Lys | Glu | Tyr | Lys | Cys | Lys | Val |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |
| Ser | Asn | Lys | Ala | Leu | Pro | Ala | Pro | Ile | Glu | Lys | Thr | Ile | Ser | Lys | Ala |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
| Lys | Gly | Gln | Pro | Arg | Glu | Pro | Gln | Val | Tyr | Thr | Leu | Pro | Pro | Ser | Arg |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |
| Asp | Glu | Leu | Thr | Lys | Asn | Gln | Val | Ser | Leu | Thr | Cys | Leu | Val | Lys | Gly |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |
| Phe | Tyr | Pro | Ser | Asp | Ile | Ala | Val | Glu | Trp | Glu | Ser | Asn | Gly | Gln | Pro |
|     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser  
 420 425 430

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln  
 435 440 445

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His  
 450 455 460

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys \*  
 465 470 475

0933916 092699